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HERITABILITY OF CONFORMATION IN POLAND-CHINA SWINE AS EVALUATED BY SCORING[1]

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THE object of the present study was to measure the heritability of visible differences in conformation among the swine in the Poland-China herd at the Iowa Agricultural Experiment Station. This herd constitutes a part of the Regional Swine Breeding Laboratory. Heritability may be defined as the fraction of the observed variance which is caused by differences in heredity. When the degree of heritability of a characteristic is low, the rate of progress which can be expected to result from selecting as breeding stock those animals which conform most closely to the ideal is correspondingly low. Whether heritability is high or low tells nothing about how far the average conformation can ultimately be changed by selection. But heritability is one of the two main factors determining the rate of change that can be made per generation, the other being the intensity of the selection which can be practiced.

Conformation was evaluated by experienced judges, usually five, who used a scoring scheme being tried in the Regional Swine Breeding Laboratory. The pigs are scored on six different items when they are near 225 pounds in weight. These items are health and vigor, quality, length of body, details of conformation, animal as a whole, and market grade. Each item may range in score from 0 to 9, nine being a perfect score. Thus 54 is the highest possible total score. It is intended that differences in the total score shall be proportional to differences in the desirability of the pigs to the butcher, modified by some attention to such things as crooked legs, hernia, etc. which would not matter to the packer but would be important to the hog producer. Information concerning how well the scoring system actually does what it is intended to do as a measure of live animals for the desired economic characteristics is meager. However, Molln (1939) has reported a correlation of .61 between score and 180 day weight. He also found a correlation of .31 between the live-score and carcass-score. We found in the present study that the number of pigs a sow raised to 225 pounds tended to increase by .05 for each increase of one point in the sow's own score as a gilt. This sort of information indicates that when the animals with the higher scores are selected there is some tendency to obtain animals that grow faster, yield better carcasses, and produce more pigs than those with lower scores.

[1] Journal Paper No. J-944 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 32. This study was conducted in cooperation with the Bureau of Animal Industry of the United States Department of Agriculture.

[2] Research fellow and professor of animal breeding, respectively.

The Causes for Variations in Scores

Fisher's analysis of variance as described by Snedecor (1940) was employed in this study in measuring the average amount of variance which arose from four general groups of causes, each of which may have included both environmental and genetic effects: (A) Differences in mean score of the herd from season to season; (B) differences in the mean score of the progeny of various sires within the same season; (C) differences in the mean scores of various litters by the same sire; and (D) differences in scores of litter mates.

TABLE 1.—ANALYSIS OF THE VARIANCE IN PIG'S SCORES DUE DIFFERENCES IN SEASONS, SIRES, DAMS, AND LITTER MATES

Source of variation	Degrees of freedom	Mean square	Composition of mean square	As a percentage of A+B+C+D
Total	1459	25.71		
Between seasons	5	544.36	$D+\frac{1460}{300}C+\frac{1460}{71}B+\frac{1460}{6}A$	A = 7.2
Between sires within seasons	65	89.95	$D+\frac{1460}{300}C+\frac{1460}{71}B$	B=10.6
Between contemporary litters by the same sire	229	33.01	$D+\frac{1460}{300}C$	C=11.5
Within litters	1160	18.44	D	D=70.7

Table 1 is a summary of that. A, B, C, and D are all expressed in terms of how much the variance between individual pigs is increased by a difference of the kind indicated. The differences between seasons, between sires, and between litters were all highly significant, statistically.

D is the average variance within litter. Besides genetic differences between litter mates, it includes the effects of environmental differences between them and the effects of any errors or clumsiness in the scoring technique which might cause the differences between the scores of litter mates not to be proportional to the actual visible differences between them. C = 2.99 and, besides the extra genetic differences brought about by the pigs being only half sibs instead of litter mates, includes the effects of whatever environmental differences existed between litters but not between litter mates. This would include any effect of variations in the health of the dam during gestation, any peculiarities in the dam's nursing ability, etc. B = 2.77

and, besides the extra genetic difference which comes from their being non-sibs instead of paternal half-sibs, contains whatever genetic differences there were between lines. These may have been considerable in the present data, since each line was bred within itself and the inbreeding in the various lines was already high enough (8 to 45%) to make it likely that they would be genetically somewhat different from each other. $A = 1.87$ and includes the effects of any changes in the average environment from season to season, any genetic changes in average score which may have occurred, and any changes which may have occurred in the standards according to which the judges did the scoring.

Intensity of Selection for Score

The sires of the pigs averaged 3.92 points higher in score than the average of the entire pig crop in the season in which they were born, while the dams averaged 3.09 points higher than the average. The intensity of selection for score could, of course, have been higher if nothing else had been considered. The score itself constituted about one-fifth of the total index upon which selection was based. Score was also highly correlated with 180 day weight, which constituted about two-fifths of the total index.

Estimating Heritability

Intra-sire correlation or regression of offspring on dam appears to be the method of estimating heritability most applicable to these data, since the dams were a selected group (Lush, 1940). Such a regression coefficient simply shows the average difference in score between paternal half-sibs for each unit by which their dams differed in score.

Doubling such a regression in accordance with Johannes Schmidt's principle of "diallel crossing" gives an estimate of the fraction of variance caused by the average effects (the additive effects) which the genes have individually in that population. This estimate also includes a small amount of the variance caused by epistatic interactions, since some of the gene combinations, especially the simpler ones, which produce these special effects would by chance at segregation be transmitted intact in a small proportion of the gametes. That is, the definition and method of analysis here have classed as non-hereditary the differences between dams which were caused by dominance deviations from the additive scheme, and most of those caused by epistatic deviations. Such differences could logically be considered as "hereditary" in the broad sense which includes all the actual effects of the genotype as a whole, although they are not hereditary in the narrow sense used here which considers the genotype as the sum of the average "good" and "bad" effects of all its constituent genes.

The estimate of heritability may contain sampling errors because of the part which chance plays in Mendelian segregation, and also because the impact of environmental factors may not have been exactly balanced as between the high and the low dams, or between their offspring. So far as these errors were random, increasing the amount of data tends to make them unimportantly small. Errors from Mendelian sampling are independent and random from pig to pig. Errors from environmental variations were, as far as we know, random from litter to litter but not wholly so between litter mates. Litter mates would be subject to some environmental circumstances which would apply alike to the pigs in that litter but not to pigs in other litters. Also a dam was scored but once. Increasing the number of her pigs would in no way cancel or diminish the effects of any mistake or environmental influence which may have made her score too high or too low. Hence the sampling errors of the present estimates are more nearly determined by the number of sows than by the number of pigs. For computing the regression coefficient each pig was considered as a unit, but in testing statistical significance the degrees of freedom were based on the number of sows.

Regression of Pig's Score on Its Dam's Score

This analysis concerns the scores of 1460 pigs farrowed during six seasons from the spring of 1938 to the fall of 1940, and the scores of their dams. The pigs were sired by 71 boars and from 300 different litters. Of the 300 litters, 205 were out of sows which produced but one litter, 80 were out of sows which produced two litters each, and 15 were from sows producing three litters. This small amount of duplication of dams was not thought to be important enough to exclude those litters from the analysis, since the second litters in all cases were subject to somewhat different environment and in nearly all cases were only half-sibs to the previous litter. They therefore went into different intra-sire populations. Table 2 shows a summary of the results.

The regressions varied rather widely from one season to another and still more widely from sire to sire within a season, but much of this variation (perhaps all of it) was due to sampling errors. These naturally would be large when the intra-sire groups consisted of only a few dams. On the average an increase of one point in the score of the dams led to an increase of one-tenth of a point in their offspring's scores. If one-tenth is the real value of the regression, statistical significance at the .05 level would be attained only with a volume of data about twice that available for the present study. Since the regression coefficient represents only half the genetic value, the total genetic variance of score in the population would be twice the regression coefficient or 20 per cent. But the fiducial limits of this (at the $P = .05$ level) would be zero and about 44 per cent.

TABLE 2.—REGRESSION OF OFFSPRING SCORES ON DAM SCORES BY SEASONS, 1938–1940. (S=SPRING PIGS; F=FALL PIGS)

Season farrowed	No. pigs	No. sires	No. dams	Intra-sire regression coefficient	Ave. inbreeding of population[1]	Ave. offspring scores	Standard deviations of scores
1938S	253	10	52	−.13	20.0	36.0	3.9
1938F	53	4	10	.20	22.8	35.6	5.2
1939S	417	16	85	.08	20.6	37.9	4.8
1939F	154	6	27	.35	20.2	36.0	5.1
1940S	353	19	77	.06	22.3	39.5	3.8
1940F	230	16	49	.42	20.6	38.4	5.7
Total or Average	1460	71	300	.10	—	38.0	5.1[2]

[1] In percentage, based on pigs at weaning.
[2] Intra-season.

This estimate concerns only the intra-line variance, since each boar's mates belonged to the same line as he did. Therefore these are percentages of a variance consisting of D plus C plus part but not all of B in table 1. The genetic variance would thus have a numerical value of between 4.3 and 4.8 in units of score, if 20 per cent is the true value for heritability.

Discussion

If only about 20 per cent of the variation in the score of swine at 225 pounds is due to the additive effects of genes, many mistakes will be made when selecting animals which are thought to have genes for superior conformation. Progress in improving the conformation of a population of swine through individual selection will be slow. The actual improvement in conformation in the next generation would be about 20 per cent of the amount which was reached for when selecting the parents.

It might be that the highest scoring pig is an intermediate in many visible characteristics. That is, we may not want the rangiest pigs, nor yet the shortest ones. For characteristics where this is the case, many of the genes act epistatically in their effects on score even though they may act additively on the anatomical units of the animal, such as length of the vertebral column or of the legs. The breeding stock in the herd in 1937 when these data began to be collected had been the result of several generations of somewhat the same kind of selection (but less systematic) as was practiced since then. If the mean number of plus genes at that time averaged close to the desired number, we would expect the offspring of the dams with the higher scores to be spreading out constantly in both directions toward the less-desired extremes. Selection for an intermediate characteristic under such

conditions would not consistently change the frequency of any one gene but would merely change temporarily the gametic ratio by eliminating more of those potential parents which would produce a high proportion of extreme gametes (Wright, 1935). This is a *possible* explanation for heritability of score being no higher but need not yet be considered as demonstrated in this particular case.

Since the pigs in this study were inbred enough that one-fifth of the original variance in score should have been lost, the heritability figure needs adjustment if it is to be applied directly to outbred pigs. On the basis of this adjustment we would expect about 24 per cent of the variance in the scores of purebred (but not inbred) Poland-China swine to be due to genetic differences, if our original foundation stocks were reasonably representative of the breed as a whole.

The scores have tended to rise slightly during the six seasons studied here, the last three seasons averaging 1.5 points higher than the first three. This is an average interval of almost one generation. It cannot be considered certain that all of this change is due to genetic improvement. The scoring standards, being partly subjective, may have shifted. However, with a heritability of 20 per cent and with parents scoring 3.55 points higher than the average of the population in the season in which they were raised, we would have expected the average score of the pigs to increase .71 point per generation if selection had been primarily for score, and somewhat more if a considerable part of that 3.55 resulted indirectly because of the correlation of score with 180 day weight for which selection was practiced directly. The time interval is much too short to use the trend in average score as a test of the heritability figure found but, so far as they go, results and expectation are in reasonable agreement.

Summary

1. An estimate has been made of the heritability of variations in the conformation of Poland-China pigs as measured by a scoring scheme being tried by the Regional Swine Breeding Laboratory.

2. The average score in this study of all of the pigs was 38.0 with a standard deviation of 5.1. The total variance in scores was partitioned according to source as follows: season-to-season changes in the herd average, 7 per cent; differences between the progeny averages of contemporary sires, 11 per cent; differences between litter averages, 11 per cent; and differences between litter mates, 71 per cent.

3. The intra-sire, intra-season regression of offspring's scores on dams' scores was +.10. Doubling this yields an estimate that twenty per cent of the variation between the scores of gilts which were later mated to the same boar was due to differences in the additive effects of their genes.

4. With the differences in scores being 20 per cent heritable and with the parents scoring 3.55 points higher than average, it can be expected that the average score of population would be increased about .71 point per generation.

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GENETIC RESISTANCE TO BRUCELLOSIS IN SWINE

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IN THE course of investigations on the epidemiology and course of contagious abortion in swine due to *Brucella suis*, two sows and a possibly related boar could not be infected—as determined by the agglutination test—by administering the organism by way of mouth. In addition, it was not found possible to infect the boar by subcutaneous inoculation with the strain of *Brucella* used to vaccinate cattle. Although these were grade stock and their pedigrees unknown, they were out of stock that originated in the University herd, and there was evidence that they were related to each other. Since the possibility of an hereditary resistance presented itself, a breeding program was initiated to determine if such resistance could be transmitted to their progeny. This paper is a progress report on these investigations. A preliminary report (Cameron, Hughes and Gregory, 1940) and a report on the bacteriology (Cameron, Gregory and Hughes, 1941) have been published, but are reviewed briefly in this paper.

Methods

Progeny were weaned at eight weeks of age and the organism, a virulent strain of *Br. suis* that had been isolated from an outbreak of abortion in swine, administered two weeks later. The organism was subjected to periodic guinea pig passage to determine if virulence were being maintained. A 24-hour culture was suspended in saline to a density of Gates 4. Five cc. of the suspension were administered orally, using a dose syringe with a spray nozzle.

Prior to, and three weeks following administration of the organism, the pigs were blood-tested for the presence of agglutinins against *Brucella*. The tube technique with dilutions of 1:25, 1:50, 1:100, 1:200, was used. Thereafter monthly tests were conducted and resistance or susceptibility determined at the completion of three tests. The absence of agglutinins (a negative test) indicated resistance, their presence (a positive test) indicated susceptibility. A consistent reaction in the highest dilution was considered positive, and a consistent absence of agglutinins, or their transient presence in the lower dilutions only, negative. Intermediate reactions were classified as undetermined until their true status could be determined.

Controls on the method of administration consisted of a group of 34 pigs of comparable age being consigned to a feed lot. These were handled in a manner similar to the selected progeny. As a control on the age factor, a

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second attempt to infect resistant animals was made. To control the criteria that determined resistance and susceptibility and to ascertain if the genetic factor were an inability to produce agglutinins rather than an ability to resist the organism, bacteriological examinations were made on a number of pigs at autopsy. In these examinations the pharyngeal, bronchial, gastrohepatica, and mesenteric lymph glands and spleen were aseptically removed at autopsy, cultured, and injected into guinea pigs. In addition, vaginal swabs were taken from all sows at farrowing to ascertain if the organism were being eliminated at that period.

Results

Table 1 shows the results of attempts to infect the two sows (47 and 59) and the boar (4). The sows were consistently negative to the agglutination test whereas the boar showed a consistent reaction in the lower dilutions

TABLE 1.—RESULTS OF ATTEMPTS TO INFECT TWO SOWS AND A BOAR WITH *BRUCELLA SUIS* (I = INCOMPLETE REACTION)

Animal (No. and sex)	Dates infected	Agglutination reaction	
47 (female)	April 29, 1938	May 20, 1938	— — — —
		July 2, 1938	— — — —
	October 21, 1938	October 21, 1938	I — — —
		November 3, 1938	— — — —
		May 1, 1939	— — — —
	November 3, 1939	November 27, 1939	— — — —
		December 27, 1939	— — — —
		January 29, 1940	— — — —
59 (female)	January 1, 1938	February 14, 1938	— — — —
		March 28, 1938	— — — —
		May 29, 1938	— — — —
	October 21, 1938	November 3, 1938	— — — —
		January 5, 1939	— — — —
		May 1, 1939	— — — —
	November 3, 1939	November 27, 1939	— — — —
		December 27, 1939	— — — —
		January 29, 1940	— — — —
4 (male)	February 2, 1938	February 21, 1938	+ I — —
	Bovine strain, 5 cc.	March 7, 1938	— — — —
	subcutaneous	July 5, 1938	I — — —
	October 21, 1938	November 3, 1938	+ I — —
	Oral. Swine strain	November 21, 1938	+ — — —
		May 1, 1939	+ I — —
	November 3, 1939	November 27, 1939	I — — —
		December 27, 1939	I — — —
		January 29, 1940	— — — —

that made classification difficult. Although definitely not susceptible, he did not exhibit the degree of resistance shown by the sows.

Table 2 shows the results of the attempt to infect the 34 control pigs (unselected population) and compares them with a similar attempt on the first 33 experimental progeny (selected population), all of which were sired by the same boar.

The marked difference in the agglutination reactions in the two groups indicates that the oral method of administration was effective.

TABLE 2.—RESULT OF ATTEMPT TO INFECT UNSELECTED STOCK COMPARED WITH RESULT OF A SIMILAR ATTEMPT ON SELECTED STOCK

Types of animals used	Number of animals	Number of animals negative	Agglutination reaction of positive animals	
			1:25	1:50
Unselected population	34	3	31	12
Selected population	33	24	9	4

The second attempt to infect included 30 animals, all of which were taken from the 33 shown under selected population in table 2. In only one did the status change from resistant to susceptible, an indication that an age factor was not involved.

The results of the bacteriological examination (Cameron, Gregory and Hughes, 1941) of pigs autopsied at various periods following administration of the organism indicated from the correlation of the blood test and bacteriological findings that the resistance was an ability to resist the organism and not an inability to produce agglutinins against it. Positive cultures from the submaxillary lymph glands of three animals in the resistant group one and two months after attempts to infect is evidence of the effectiveness of the method of administration in that invasion of the tissues had occurred. It also indicates, since cultures made more than two months after attempts to infect were consistently negative, that the resistance of the host prevented further invasion. Additional support for the conclusion that the host is resistant lies in the fact that in all farrowings of resistant sows, a total of 55 to date, none has been found eliminating the organism at farrowing.

Resistance or susceptibility could not be determined definitely in some progeny because of inconclusive reactions to the blood test. These animals are tentatively grouped as undetermined. Although numbers in this group were too few to be conclusive, a trend towards resistance was indicated.

To date, 23 litters, totaling 128 progeny, have been tested for resistance and susceptibility. These are divided into four group matings. All females

bred were resistant. One group of eight litters resulted from mating to boar 4, the original boar that showed a reaction in the low dilution only. A second group of eight litters resulted from mating to a completely resistant boar; a third of two litters from a brother-sister mating of resistants; a fourth of five litters from mating to a resistant pure-bred Duroc-Jersey boar (No. 33). The results shown in table 3 indicate that resistance is genetic. The original resistant animals were obtained from matings of proven susceptible animals. This suggests that resistance may be recessive, but it does not preclude the possibility of a dominant mutation for resistance. If resistance is recessive, all the progeny from resistant times resistant matings should be resistant. On the other hand, if resistance is dominant and monofactoral, one should expect three-fourths of the progeny to be resistant and one-fourth susceptible from heterozygous matings.

Approximately 77 per cent of the progeny are resistant, one animal (or 0.8 per cent) is susceptible and the remaining animals, constituting almost 23 per cent, are undetermined (table 3). Because of the animals in the undetermined class, it is rather difficult to interpret the results presented in table 3. If the animals classified as undetermined eventually prove to be genetically resistant the conclusion that resistance is recessive, but that an occasional resistant animal becomes infected, would seem tenable.

TABLE 3.—THE RESULTS OF TESTS OF PROGENY FROM RESISTANT × RESISTANT MATINGS, BASED ON 23 LITTERS TOTALING 128 PIGS (F = FEMALE, M = MALE, R = RESISTANT, S = SUSCEPTIBLE, U = UNDETERMINED)

Matings	Progeny									
	No. of litters	Resistant		Susceptible		Undetermined		Percentage		
F M		F	M	F	M	F	M	R	S	U
R. × 4	8	18	14	0	0	4	6	76.2	0	23.8
R. × R. 9	8	26	20	0	0	3	4	86.8	0	13.2
R. 13 × R. 9*	2	1	2	0	0	2	4	33.3	0	66.7
R. × R. 33	5	8	9	0	1	4	2	70.8	4.2	25.0
Totals	23	53	45	0	1	13	16	76.6	0.8	22.6

* Brother-sister mating.

On the other hand, if all the animals of the undetermined group (approximately one-fourth) prove to be genetically susceptible, it would not prove that resistance is dominant and monofactoral since many of the litters in table 3 could not be from heterozygous parents. If one assumes that resistance is dominant, the matings in table 3 could be of three types:

AA×AA, AA×Aa, and Aa×Aa, the last two types of mating probably being more numerous. It is apparent that if one assumes resistance to be dominant, there are too many susceptible animals (here it is assumed that the undetermined animals of table 3 prove to be genetically susceptible).

Since conclusive data are not available, the exact mode of inheritance of resistance must be regarded as an open question. A working hypothesis that is consistent with the present data is that resistance is recessive and monofactoral with a penetrance of about 77 per cent. Additional data should prove or disprove this hypothesis.

From a genetic standpoint, the animals in the undetermined group and the one exceptional resistant animal are of great interest and importance. This genotype should be checked against the agglutination test.

Discussion

The ease by which swine are infected with *Brucella suis*, the fairly efficient method by which infection can be diagnosed, and the high prolificacy make this animal a practical subject for investigating disease resistance. Although Brucellosis in swine can be controlled by blood testing and segregation or slaughter in a manner similar to that used in cattle, the use of resistant strains in the control of the disease has a practical application. A genetically resistant stock has not been utilized in the control of disease among livestock to the extent that it has in plant diseases. The majority of the work in livestock has been with poultry where it probably has more application than in other domestic animals. Roberts and Card (1926) have demonstrated a genetic resistance to pullorum infection in poultry. This disease resembles Brucellosis in swine in that both are bacterial infections, diagnosed by serological tests, and controlled by segregation or disposal of infected stock.

The investigations reported have been confined to grade stock. Search, however, has been made for the resistance in pure-bred stock in the University herd where the foundation stock of the present pigs originated. Resistance has been demonstrated in the Poland-China and in the Duroc-Jersey, the only breeds tested so far. Breeding tests are under way with the object of establishing resistant strains in herds where the disease is enzootic. It is under field conditions that the resistance must finally be tested.

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THE EFFECT OF SEX ON THE DEVELOPMENT OF THE PIG. II. URINARY EXCRETION OF ANDROGENS BY BOARS OF DIFFERENT LINES OF BREEDING[1]

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ANDROGENIC substances have been found in the testes and urine of many of the farm animals. Ssentjurin (1926) perfused bull and stallion testes and recovered substances which would condition the capon's comb and produce the secondary sexual characteristics of the cock. Womack and Koch (1932) reported that male hormone was found in ram testes and that some seasonal fluctuations in the testicular content were to be found. Androgenic substances were recovered from boar testes by Ogata and Hirano (1934), and Hanes and Hooker (1937) found some difference in the androgen content of normal and cryptorchid boar testes.

Androgens were found in bull and ram urine by Butz and Hall (1937). The potency of various urines has been tabulated by Gustavson (1939). Although the estrogen content of testes or male urine has not been studied as extensively as testicular or male urinary androgens, Zondek (1934) found large amounts of female hormone substance in both the urine and testes of the stallion.

Materials and Methods

Very noticeable differences in the time of puberty and virility have been observed in various lines of inbred swine developed at the Minnesota Experiment Station. Studies of the urinary androgen and estrogen excretion by boars of some of the lines are being made as the initial study of the possible causes of the variations in breeding behavior.

For the work reported in this paper, a total of 105 48-hour urine samples were collected. The urine was secured by placing the boars in screen-bottomed crates. A pan was placed below the screen in such a position that the back edge of the pan was far enough posterior to the prepuce to catch all of the urine and yet allow the feces to fall clear of the pan. By frequent removal of feces which might collect in the screen, care in feeding and watering, and by careful brushing of the animals before use, the urine samples were kept quite free from contamination. The urine was collected in glass

[1] Paper No. 1939, Scientific Journal Series of the Minnesota Agricultural Experiment Station in cooperation with the Regional Swine Breeding Laboratory, Ames, Iowa, Bureau of Animal Industry, U. S. Department of Agriculture.

[2] Assistance in the preparation of this material was furnished by the personnel on W.P.A. Official Project No. 165-1-71-124, Subproject 495, sponsored by the University of Minnesota in 1941.

bottles under toluene. The samples were stored in a refrigerator at 5° C. until used. All volume measurements were made immediately after removal of the urine from the refrigerator.

The techniques outlined by Gallagher *et al.* (1937) were followed for hydrolysis (15 minutes) extraction and separation of the androgenic and estrogenic fractions. The extractors used were of an improved type. Capon comb growth assays (Gallagher and Koch, 1935) were made and table 1 of Gallagher *et al.* (1937) was used for calculation of the results. Colorimetric assays were made by use of an Evelyn type colorimeter and the technics of Callow *et al.* (1938) and Holtorff and Koch (1940) were followed. The latter technic was used for all colorimetric assay results given below.[3]

Results

The range in the amount of urine per 48 hours was none to 15,600 ml. (av. 5,715 ml.). The color varied from a light lemon to a dark reddish brown and a slight turbidity was noted in almost all samples.

One estrogenic sample was assayed by use of the mouse vaginal smear technic and the sample (48 hours) was found to contain 71.45γ estrone equivalent.[4]

A correlation coefficient of 0.8 ($P < .01$) was found between the capon and colorimetric assay technics. Sixteen assays were used for calculating the coefficient.

The first trial was conducted to establish, if possible, any differences in the amount of androgen excreted by individuals. Two boars were used and assays were made by alternating capons used for injections. The amount of comb growth obtained was as follows:

Boar 372 (C.S.) 96-hour urine sample,[5] growth = 12.5 mm L+H
Boar 372 (C.S.) 48-hour urine sample, growth = 6.0 mm L+H
Boar 381 (C.S.) 96-hour urine sample,[5] growth = 8.5 mm L+H
Boar 381 (C.S.) 48-hour urine sample, growth = 4.5 mm L+H

The growth secured from the samples indicated that No. 381 excreted androgens at a lower ($\frac{2}{3}$) rate than No. 372.

A second experiment was initiated to extend the observations noted above and to test possible differences between boars of various lines of breeding. Assays were made in capon units and the results are given in table 1.

[3] The authors are indebted to Dr. Erwin Schwenk of the Schering Corporation for their generous supplies of androsterone for capon assays and colorimeter standardization.

[4] The authors are indebted to Dr. L. T. Samuels and Miss Clara M. Szego of the Department of Physiological Chemistry, University of Minnesota, for this assay.

[5] Ninety-six-hour sample represents the combination of two 48-hour samples. Peanut oil was used as a vehicle in all capon assays.

TABLE 1.—URINARY ANDROGEN EXCRETION BY BOARS OF DIFFERENT GENETIC BACKGROUND

(Assays were made in capon units per 48-hour urine sample)

Boars, line I			Boars, line II	
No. 381	No. 550	No. 562	No. 672	No. 926
.36	.21	.82	.24	1.59
.74	.66	.54	1.51 1.49	2.09
Av. .55	.44	.68	1.08	1.84
Av. line I, .556			Av. line II, 1.38	

Individual differences in the amount of androgen excretion were again indicated as well as possible "line" differences.

A third trial was conducted to test age and line differences. Two boars of line I, three of line III, and two boars representing a cross of lines I and II were used for the study. Samples were secured at approximately weekly intervals from December, 1940 to March, 1941, and the age range was 10–30 weeks. The results were as in Table 2.

TABLE 2.—URINARY ANDROGEN EXCRETION (PER 48 HOURS) BY BOARS OF VARIOUS LINES OF BREEDING[1]

Age (weeks)	Line of breeding		
	I	III	I×II
10–11	—	1850(2)	—
12–13	1638(3)	—	1979(1)
14–15	1734(2)	3636(3)	1750(3)
16–17	2494(3)	2971(4)	2487(3)
18–19	1665(3)	5041(5)	2487(3)
20–21	2789(2)	5723(3)	3034(4)
22–23	2373(4)	3260(2)	2112(3)
24–25	2193(2)	7646(4)	—
26–27	—	5217(4)	—
28–29	—	3380(2)	—
30–31	—	1408(1)	—

[1] Results are given in gamma androsterone equivalent as determined by colorimetric methods (620 filter). The numbers in parentheses refer to the number of samples averaged. The averages for the various lines were: line I, 2,122γ; line III, 4,523γ; and line I×II, 2,368γ.

One mature boar of line II averaged 3,255γ androsterone equivalent for three samples.

In addition to these samples, 20 other boars of these lines of breeding from whom 170 samples have been secured are giving similar results.

Discussion

Boars vary in their androgen excretion and the genetic background of these boars seems to have some influence on their urinary androgen discard. Individuals of line III excrete much more male hormone than those of line I or I×II. However, until a larger sample of the lines may be taken, it would be difficult to assign observed differences in sexual behavior to these variations in androgen excretion only. Although the expression of sex impulse is many times associated with the activity of the testes secretion alone, the sex impulse, as with many other body functions, is not necessarily the result of only one causative agent. For example, the role of the pituitary and its action on the gonad is generally known. In some cases lack of sex urge is not basically caused by testes hypofunction but by dysfunction of the pituitary body. Likewise, the thyroid and probably other glands of internal secretion play a role in this expression. Therefore before a complete answer may be given to the causes of different degrees of sex impulse, correlated studies of these factors as well as testes activity must be made.

Summary and Conclusions

1. Androgenic substances have been found in boar urine.

2. These substances may be assayed by the usual capon comb growth technic or by colorimetric methods.

3. A correlation coefficient of 0.8 ($P < .01$) was found between these two assay methods.

4. Boar urine also contains estrogenic substances.

5. Variations were found in the amount of androgen excreted by different individuals and by boars of different lines of breeding.

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THE MINIMUM REQUIREMENT OF PANTOTHENIC ACID FOR THE GROWING PIG[1]

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Introduction

THAT pantothenic acid is necessary for normal growth and well being in the pig was demonstrated by the authors in 1940–41.[3] A highly purified diet had been used successfully at this station in determining the minimum requirement of thiamin (Hughes, 1940*a*), and riboflavin (Hughes, 1940*b*), for this species and inasmuch as (dl) calcium pantothenate was available it was decided to determine if possible the minimum requirement of this factor (pantothenic acid) for the grow..ng pig.

Experimental Procedure

From former experiments there was some evidence that the pantothenic acid requirement was probably higher than for thiamin or riboflavin. Four groups of pigs (5 in each lot) were fed a highly purified diet with pantothenic acid at varying levels of 0, 4, 8 and 12 mg. per 100 pounds of pig daily. The lots of pigs were uniform in age and weight. The average beginning weight was slightly more than 30 pounds each. They were quartered in similar pens with concrete floors, each having an inside and an outside pen where sunlight and shade were available. They were fed and watered in metal troughs.

The ration for a week was mixed in a clean metal feed box and then placed in separate metal cans which were lined with paper. The vitamins (see next paragraph) were weighed carefully, put into water solution, then mixed with casein, which in turn was thoroughly mixed with the other components of the diet. All of the pigs were fed twice daily and each was weighed separately each week.

Diets Fed

Lot 1 was fed the basal ration which was composed of the following: Granulated sugar 81%, casein (purified) 15%, salt mixture 4%. In addition the following synthetic vitamins were fed in milligrams per 100 pounds of pig daily: Thiamin 3 mg., riboflavin 4.5 mg., nicotinic acid 15 mg., pyridoxine (vitamin B_6) 5 mg. Vitamin A was provided in the form of codliver oil (Squibbs tested) which was given at the rate of 10 cc. per pig weekly.

[1] The experimental work reported in this paper became cooperative with the United States Bureau of Animal Industry, July 1, 1938. The work was also aided by a grant from the American Dry Milk Institute, Inc.

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[3] In process of publication.

Lot 2. Fed the basal ration plus 3.6 mg. of pantothenic acid per 100 pounds of pig daily.

Lot 3. Fed the basal ration plus 7.8 mg. of pantothenic acid per 100 pounds of pig daily.

Lot 4. Fed the basal ration plus 11.8 mg. of pantothenic acid per 100 pounds of pig daily.

The salt mixture used was made up of the following salts: Calcium phosphate (dibasic) 40%, sodium chloride 20%, calcium carbonate 11%, ferric chloride 3%, copper sulphate 0.2%, manganese sulphate (anhydrous) 0.5%, zinc oxide 0.1%, magnesium citrate 15%, cobaltous acetate 0.1%, potassium chloride 10%, and potassium iodide 0.1%.

Results

The experiment began May 27, 1941 and closed July 29, 1941, covering a period of 63 days. Table 1 and figure 1 show graphically the results obtained.

TABLE 1.—SUMMARY OF AVERAGE WEIGHTS, AVERAGE DAILY GAINS, AND (dl) CALCIUM PANTOTHENATE FED

Lot number	Average initial weight	Average final weight	Average daily gain	Feed consumed per 100 pounds of gain	Pantothenic[1] acid per 100 pounds of pig daily	Calcium pantothenate (dl) per 100 pounds of pig daily
	pounds	pounds	pounds	pounds	mg.	mg.
1	30.6	34.2	0.06	1183	0.0	0.0
2	30.6	55.0	0.4	284	3.6	7.9
3	30.6	58.2	0.4	278	7.8	17.1
4	30.6	68.0	0.6	244	11.8	25.8

[1] Only the dextro-rotatory form of calcium pantothenate is biologically active. One gram of (dl) calcium pantothenate is therefore equivalent to 0.5 gm. of dextro-rotatory calcium pantothenate or to 0.46 gm. of dextro-rotatory (natural) pantothenic acid.

Pigs in lot 1 made an average gain of less than four pounds for the 63-day feeding period. During the whole time they were difficult to keep on feed, and all of them scoured and vomited from time to time throughout the trial. After 23 days some of the pigs started to pass blood in their feces and this continued intermittently throughout the trial. All of the pigs in this lot passed some blood but two individuals were more persistent than the other three. As the test progressed the pigs became more and more emaciated; their hair became dry and very rough. Two of the pigs were kept on this diet for two months and they showed some loss of hair over the back, shoulders, and rump.

The most characteristic external manifestation of pantothenic acid deficiency is the peculiar gait that developed in about 50 days. As they walk the

pigs do an extreme "goose step" with their hind legs; while standing they often have a rhythmic kicking with one hind leg and then with the other. This condition develops gradually and the two pigs kept on the diet after the trial could not move without showing some form of "goose step" action.

One pig died after having been on the experiment for 43 days. An autopsy

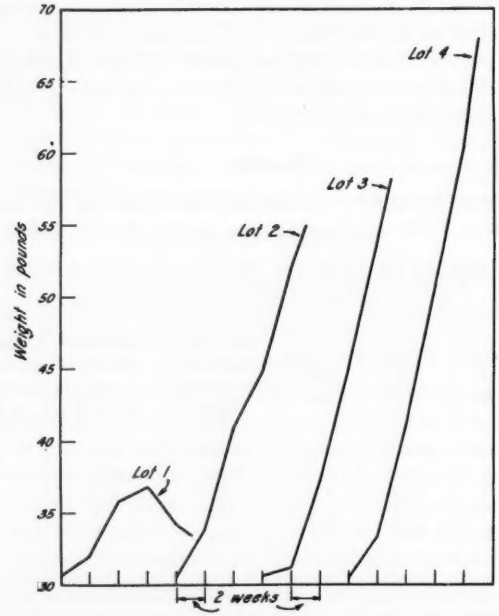

Figure 1. Growth curves of pigs fed varying amounts of pantothenic acid.

showed the whole intestinal tract and stomach to be inflamed with many signs of hemorrhage; however, no ulceration had developed. Another pig was killed and autopsied at the close of the trial and it showed a little gastritis in the stomach and some congestion and a few ulcers in the caecum.

Lots 2 and 3 had very similar growth rates; their average gain was about 27 pounds per pig. Pigs in lot 2 showed all the conditions found in lot 1 but to a lesser extent. The pigs in lot 3 scoured, passed a little blood, "goose stepped" occasionally, and their hair seemed dry. However, they were definitely more thrifty and in better condition at the close of the trial than pigs in lot 2.

At the end of the trial one pig from lot 1 and two pigs from lot 2 were fed

the diet of lot 4. Within 21 days these three pigs showed a decided improvement in their walk and appetite, and their rate of gain increased from 0.4 to 1.0 pound each per day. Autopsy of three pigs in lot 2 showed small congested areas in the stomach and some congestion in the large intestine, particularly in the caecum. The small intestine appeared normal.

The pigs in lot 4 had an average gain of 38 pounds for the test period, appeared normal, and showed none of the symptoms of those in the other pens. All the pigs appeared thrifty, although their hair was a little dry; their legs were strong, and their action was good. Two hogs were autopsied at the close of the trial and all their internal organs were normal in appearance.

Discussion

A lack of pantothenic acid in the diet of the pig causes poor growth, a rough dry coat, and affects the digestive tract, particularly the stomach and the larger intestine. Congested areas, hemorrhagic areas and ulcers may be found. Wintrobe, *et al.* (1940), described a gait in pigs that is similar to the one discussed here. They did not specify the dietary factor causing this condition but they did study and describe the nerve changes that took place preceding the condition.

A study of the growth curve of the four lots of pigs indicates the minimum requirement of pantothenic acid for the growing pig to lie between 7.8 and 11.8 mg. per 100 pounds of pig daily.

Summary

Using rate of growth and other symptoms of pantothenic acid deficiency as the criteria the minimum requirement of pantothenic acid lies between 7.8 and 11.8 mg. per 100 pounds of pig daily.

Acknowledgments

A number of colleagues were helpful in the course of this experiment. We wish to express our appreciation to Dr. S. H. Babcock who provided the (dl) calcium pantothenate and to Mr. Robert Squibb who fed and cared for the pigs.

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NUTRITIONAL DEFICIENCIES OF A CONCENTRATE MIXTURE COMPOSED OF CORN, TANKAGE, SOYBEAN OILMEAL, AND ALFALFA MEAL FOR GROWING PIGS[1]

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IN a series of studies dealing with the protein requirement of the growing pig, conducted during the past five years, a number of abnormal conditions (such as incoordination and lameness, growth failure, vomiting, diarrhea, inactivity, anorexia, and a mild dermatitis) have been encountered. This report deals with some observations on certain numbers of individual pigs which were fed in an effort to obtain further information concerning these disorders.

Observations on Stiffness

Among the disorders was a type of stiffness and lameness which was encountered with pigs that had gained weight unusually fast. These pigs were started on experiment at between 70 and 100 pounds live weight and were fed a mixture comprised of corn, tankage, soybean oilmeal, sun-cured ground alfalfa hay, and salt. All rations contained 4 per cent of the alfalfa meal. The quantity of the other feeds was varied to furnish rations of different total protein contents as desired. The percentage of tankage was varied from 4 to 25 per cent of the ration. Low protein bone meal tankage and 55 per cent protein digester tankage were used.

Stiffness occurred at about the time the pigs reached a body weight of 150 pounds. The condition was at first characterized by a slight halting of the gait, which slowly progressed in severity until it was only with difficulty that the pigs could rise to their feet. Their behavior indicated that the condition was painful. Most of the pigs would lie down continually and if forced to get up would not remain standing. Some of the animals would sit on their buttocks and practically all of the severely afflicted would walk on their knees. None appeared to be dizzy or showed any indication of a lack of muscular control. They continued to gain and had normal appetities and were otherwise thrifty and healthy. Their skins were clean and oily, the hair was sleek, and the eyes were clear and appeared normal in every respect with the vision unimpaired.

Thirty out of a total of 60 pigs were affected with stiffness. During the period of the experiments, 9 of the unaffected pigs did not attain the weight

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of 150 pounds. Hence, it may be said that 30 out of 51 pigs which attained a weight of 150 pounds became stiff.

In outward appearance the condition of the afflicted individuals closely resembled rickets. However, the condition was not alleviated or affected in any way by the administration of cod-liver oil or irradiated yeast. Subsequent analysis of the ulnae and radii of 18 affected pigs showed that the mineral content of these bones was normal, the average value for bone ash being 58.92 per cent on the dry, fat-free basis. Normal values were also obtained for the inorganic phosphorus content of the blood. However, there were certain constantly occurring changes observed in the leg bones which indicated abnormal development. Upon gross examination the distal extremity of the radius and of the ulna showed enlargement. Roentgenographs of this area of these bones indicate that there was an increased activity of the tissues at the oesteochondral junction, this area being definitely enlarged, but there was no apparent irregularity of the zone of ossification and no other changes present other than hypercalcification, thus differentiating the condition from rickets. The occurrence of the stiffness was first noticed during the feeding of rations which had relatively high mineral contents, ranging from 6 to 9 per cent. On analysis, these rations were found to have low manganese contents ranging from .0011 to .0014 per cent.

Manganese sulphate was then added to the swine rations in amounts to supply 50–60 parts per million of manganese. As a result, 15 pigs which received the manganese supplemented rations showed no signs of stiffness. Fifty per cent of litter and pen mates of these same pigs fed the unsupplemented rations became stiff. However, the addition of manganese to the ration was ineffective in curing stiffness after it had developed.

Observations on Further Deficiencies of the Basal Ration

In a later experiment, unusual cases of vomiting, diarrhea, anorexia, growth failure, and ataxia were observed in a group of 10 pigs fed the basal ration, presented in table I, during the winter of 1939. Five of these pigs were fed two-tenths pound of manganese sulphate with each 100 pounds of the basal ration, the rations of the remaining pigs having manganese contents of .0011 to .0014 per cent. These pigs were smaller and younger at the beginning of the feeding test than those used in the previous studies, their initial weights ranging from 26 to 40 pounds. The rate of gain of these pigs was very slow as compared to those showing the stiffness. At the end of 70 and 84 days, respectively, two pigs had died. One of these pigs had lost 5 pounds and the other had lost one pound. Both individuals had had severe spasms of vomiting, nausea, and diarrhea, they huddled together, ate very little, a mild dermatitis developed and small lesions were present on their

face and legs. These pigs were properly injected with nicotinic acid and were fed riboflavin and pyridoxine with no beneficial results.

Four of these pigs were fed the ration for a period of 147 days. Abnormal conditions were evident, but not serious. Their gains were subnormal, averaging about .5 pound per day. Cod-liver oil and nicotinic acid given to two of these pigs failed to increase the rate of gain or to improve their condition.

Another pig was fed 100 grams daily of vacuum dried pork liver meal after having a loss of body weight of 5 pounds for the first 98 days of feeding. This pig was weak and had all of the afflictions of the two pigs that died. After 63 days of feeding the liver supplement, the pig had gained 53 pounds and appeared normal in every respect.

The above facts indicate that the basal ration was deficient in one or more nutritional factors important with respect to growth, normal digestion, muscular control, and normal functioning of the skin. A study was then outlined to give further information concerning the observed deficiencies.

The ration used contained the same ingredients in similar proportions as those used in the manganese studies, table 1.

Forty pigs were selected soon after weaning at ages ranging from 48 to 60 days and weights ranging from 18 to 30 pounds.

TABLE 1.—CONCENTRATES USED IN THE BASAL MIXTURES[1]

Concentrates	Basal	Basal ration with a portion of tankage replaced by soybean oilmeal
Yellow corn (No. 2)	60.5[2]	60.5
Low protein tankage	35.0	25.0
Soybean oilmeal (expeller)	—	10.0
Ground sun-cured alfalfa	4.0	4.0
Salt	.5	.5

[1] All pigs were fed in individual stalls on concrete floors with access to concrete outside runways.
[2] Parts per 100 lbs.

The outline of the treatments was as follows:

(1) Basal and 100 grams of yeast daily. At the end of 56 days, 40 gamma of beta carotene were fed each day.

(2) Basal and 40 gamma of beta carotene daily. At the end of 56 days, 100 grams of yeast were fed each day.

(3) Basal and a vitamin mixture of nicotinic acid 75 mgm., thiamin chloride 5 mgm., riboflavin 2 mgm., calcium pantothenate 10 mgm., and pyridoxine 0.8 mgm. At the end of 56 days, 40 gamma of beta carotene were fed each day.

(4) Basal. At the end of 56 days, an alcoholic extract of whole vacuum dried beef liver was fed daily.
(5) Basal. At the end of 56 days, 100 grams of whole vacuum dried beef liver were fed each day.
(6) Basal and 100 grams of whole vacuum dried beef liver daily.
(7) Basal with 10 parts of the tankage replaced by 10 parts soybean oilmeal. At the end of 56 days, 100 grams of brewers' yeast and 40 gamma of beta carotene were fed each day.
(8) Basal with 10 parts of the tankage replaced by 10 parts of soybean oilmeal and 100 grams of brewers' yeast daily.
(9) Basal with 10 parts of the tankage replaced by 10 parts of soybean oilmeal and 40 gamma of beta carotene daily.

At the end of 56 days of feeding, all the pigs fed the basal ration showed the following symptoms: emaciation, weakness, vomiting, anorexia, diarrhea, and a mild dermatitis.

The feeding of 100 grams of brewers' yeast daily with the basal ration did not correct the deficiencies of the ration, treatment 1. Three of the 5 pigs died during the course of the feeding period; the other 2 failed to gain and exhibited the same deficiency symptoms as those fed the basal ration. After 56 days of feeding, 40 gamma of beta carotene were fed each day to these 2 pigs, with no improvement in their condition.

The feeding of 40 gamma of beta carotene daily during the first 56 days and 40 gamma of beta carotene and 100 grams of yeast with the basal ration during the remainder of the period, did not fulfill the nutritive requirements of these pigs, treatment 2.

The addition of the mixture of the B vitamins to the basal mixture did not supply the necessary essential nutrients, treatment 3. Two of the 5 pigs died; the other 3 were definitely emaciated with characteristic symptoms observed in the other pigs fed the basal ration. The feeding of 40 gamma of beta carotene each day to each of these 3 pigs, beginning at the end of 56 days, did not improve their condition.

A limited response was noted with each of the two pigs that were fed the alcoholic extract of the vacuum dried beef liver, after 56 days on the basal ration, treatment 4. The recovery of these 2 pigs was not as rapid or as complete as that of the pigs fed whole vacuum dried beef liver. Complete recovery and normal growth ensued with the 3 pigs fed the 100 grams of whole vacuum dried beef liver each day, after 56 days on the basal ration, treatment 5.

Of the 5 pigs fed the basal ration and 100 grams of the liver each day, treatment 6, three pigs died during the first 30 days of feeding. Internal hemorrhages caused the death of these pigs. The cause of the internal

hemorrhages has not been determined. The other 2 pigs made an average daily gain of .94 pound. At the end of 98 days both pigs were showing an incoordination, one pig was passing bloody urine, and a mild dermatitis was present on the ears, legs and tails of both pigs. The ataxia did not develop into a severe stage, yet there were no changes in the gaits of these animals after a subsequent 5 weeks on alfalfa pasture.

Of the 5 pigs fed the basal ration with 10 per cent of the tankage replaced by soybean oilmeal, 2 died before the end of the feeding period, treatment 7. These 2 pigs exhibited the same symptoms as those fed the basal ration. The other 3 pigs gained rather slowly. The daily allowance of 100 grams of brewers' yeast and 40 gamma of beta carotene to the 3 pigs, beginning at the end of 56 days, brought about an increase in the rate of gain during the last 49 days.

The 5 pigs fed the basal ration with 10 per cent of the tankage replaced by soybean oilmeal and 100 grams of brewers' yeast daily per pig, made an average daily gain of .60 pound with individual gains ranging from .42 to .97 pound, treatment 8. Two individuals had a mild dermatitis, and one of these had ataxia.

The 5 pigs fed the basal ration with 10 per cent of the tankage replaced by soybean oilmeal and 40 gamma of beta carotene daily, made an average daily gain of .71 pound with a range of .58 to .88 pounds for individual pigs. One pig had a moderate dermatitis, and ataxia developed at about 105 days. After feeding on alfalfa pasture for three weeks, the ataxia became more pronounced, and within a short time the animal was unable to walk, treatment 9.

Histological Studies

Histopathological studies were made on the liver, kidney, skin, and, in several cases, on the spinal cord and sciatic nerves. The skin lesions have been described as a mild dermatitis; however, it resembles more closely an epidermidosis since the lesions were confined principally to the epidermal layers. In only two cases examined microscopically did the dermatitis extend below or through the Malpighian or germinal layer. No significant pathology can be reported for the liver and kidney at this time.

Fresh sections of the spinal cord and the sciatic nerve from pigs, on treatment 9, were fixed and stained according to Marchi's technique for degeneration of the myelin sheath. Microscopic examination of this material revealed a moderate degeneration of the myelinated tracts with some fragmentation of the demyelineated nerve fibers. This was observed to a variable degree in both the sciatic nerves, the dorsal columns of the spinal cord and dorsal nerve roots. These myelin changes were not as extensive as those

reported by Wintrobe, Miller, and Lisco (1940) and by Ellis and Madsen (1941).

Summary

The addition of manganese to rations which were high in ash and low in manganese content was found to be of benefit in preventing stiffness with pigs of initial weights of 80 to 100 pounds which were gaining weight rapidly. No other deficiencies were evident with normal healthy pigs of these initial weights.

For young pigs with initial weights ranging between 20 and 35 pounds at the beginning of the experiment, rations comprised of yellow corn, tankage, alfalfa, and salt were deficient in growth factors. The factors lacking are apparently supplied by liver and a combination of soybean oilmeal, yeast, and carotene. These factors are not supplied by yeast alone or pure B vitamins.

Nutritional factors required to prevent a mild dermatitis and ataxia were not fully supplied by any of these supplements.

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THE LIMITATIONS OF OAT STRAW AS A ROUGHAGE FOR MAINTENANCE, LACTATION AND GROWTH IN SHEEP

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EVIDENCE has been accumulated that ewes maintain themselves better during the gestation and suckling periods and raise lambs of higher quality if legume hay is included in their ration than they do if oat straw is the roughage fed. Alfalfa and clover are considered to be especially good roughages for sheep, while oat straw and silage are regarded as less satisfactory. The object of the experiments reported here was to determine the deficiencies of oat straw as a roughage for breeding sheep and to correct these if possible. The results obtained are not entirely conclusive but indicate that protein is at least one of the limiting factors. Reduced milk production of ewes and slow growth and finish of lambs were the main outward manifestations of deficiency of the oat straw roughage ration.

Harper (1940) has shown, in a series of experiments over the past five years that a ration of oats, oat straw, and corn silage did not meet the requirements of gestation and lactation of breeding ewes. When alfalfa hay was fed in place of oat straw good results were obtained. Attempts to improve the oat straw ration by the addition of calcium, phosphorus, and iodine supplements were unsuccessful. The addition of soybean oil meal produced comparable wool yields but did not produce satisfactory growth and development in the lambs.

Experimental

Six lots of 15 ewes each were fed the rations described in table 1. The animals were quartered in a shed that was open on the south and which communicated with outside dry lots. The ewes were bred during October and November and after coming off of grass pasture were fed legume hay until they were placed on the experimental rations December 28, 1940. The ewes were weighed every ten days during the experimental period. All of the ewes were maintained in good condition up to lambing time, although the ewes in lot 6 which received alfalfa hay increased in weight considerably more than the others. The loss in weight of the ewes and their condition following lambing and up to the time the lambs and ewes went on pasture (May 7,

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TABLE 1.—DEFICIENCIES OF NON-LEGUMINOUS ROUGHAGES FOR BREEDING EWES AS MEASURED BY GROWTH AND FEED CONSUMPTION OF EWES AND LAMBS

Lot No.	Ration						Av. initial weight of ewes	Av. gain of ewes period of pregnancy	Av. loss in weight after partuition	Av. wool clip of ewes	No. of lambs born	No. of twin sets	Av. birth weight of lambs, twins and singles	Av. gain of lambs, pre-pasture feeding
	Ground yellow corn	Corn silage	Oat straw	Casein	Alfalfa hay	Alfalfa ash								
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*			*pounds*	*pounds*
1	.66	1.93	1.20				130.3	4.4	28.7	9.1	16	4	7.3	9.89
2	.65	1.88	1.18				128.3	2.3	34.2	8.6	16	2	7.6	13.21
3	.66	1.92	1.25	.25			132.1	6.0	26.7	9.5	16	2	8.3	22.57
4	.65	1.88	1.22	.25			128.6	9.7	22.4	9.5	15	4	7.8	19.84
5	.65	1.90	1.22			.13	129.4	2.6	26.5	9.0	12	1	8.4	10.10
6	.65	1.88			2.5		130.9	17.2	22.8	10.6	20	8	7.8	14.79

Experimental period Dec. 28, 1940 to May 7, 1941. Period of Pregnancy Dec. 28, 1940 to February 26, 1941. Post partuition period February 26 to May 7, 1941.
15 ewes in each lot.
Feed intake figures are average daily consumption in pounds.
Each ewe in lots 2 and 4 received 30,000 international units of vitamin A weekly from cod liver oil concentrate.

1941) was variable. Ewes in the alfalfa hay and casein and cod liver oil lots did not lose as much weight as those in the other lots and they were also in much better condition. The ewes in the oat straw and cod liver oil lots lost the most in weight and were in a very unthrifty condition. The other lots ranked in intermediate positions. The wool clip was the largest in the alfalfa hay lot and the lowest in the cod liver oil lot. The birth weights of the lambs were not considered significantly different, although the distribution of twins and singles in the various lots undoubtedly leaves this conclusion

TABLE 2.—MILK PRODUCTION OF EWES ON LEGUMINOUS AND NON-LEGUMINOUS RATIONS

Lot No.	Ration	No. of ewes	No. of observations	Mean day in lactation	Adjusted[1] mean daily milk per ewe	Difference in milk production
					ounces	*ounces*
6	Alfalfa hay	12	67	33.7	37.32	
						4.04*
4	Casein and cod liver oil	11	60	37.9	33.28	
						2.44*
3	Casein	14	70	40.4	30.84	
						11.81*
1	Oat straw	12	55	30.9	19.03	
						1.04
5	Alfalfa ash	11	55	25.3	17.99	
						.26
2	Cod liver oil	14	76	28.9	17.73	

[1] Adjusted to general mean day of lactation.
* Highly significant difference.

open to question. The gain of the lambs up to the time they went to pasture was highest in the casein and casein and cod liver oil lots and lowest in the oat straw and alfalfa hay ash lots. These figures are not strictly comparable because of the difference in age of the lambs and the unequal distribution of twins and singles.

In order to obtain information that could be subjected to a more critical analysis and interpretation, milk production records were made on the ewes during a seven-week period. This was accomplished by separating the ewes and lambs once each week for successive 3-hour periods during each 24 hours. Following separation, the lambs were weighed to the nearest ounce, placed with their dams and allowed to suckle, and immediately reweighed. The difference in weight was designated as the milk production for the period observed. The results, which are presented in table 2, were treated by analysis of variance and covariance. Since the lots varied significantly in

mean day of lactation, a significant regression coefficient of daily milk per ewe on day of lactation of −0.63 ounces per day was utilized in adjusting the lot mean daily milk per ewe to the general mean day of lactation. Milk production was highest in the alfalfa hay lot (6). It was significantly less in the

TABLE 3.—GROWTH OF LAMBS FROM EWES ON LEGUMINOUS AND NON-LEGUMINOUS RATIONS[1]

Lot No.	Ration	No. of lambs	No. of observations	Mean age	Mean weekly gain	Difference in gain
			Singles			
				days	*pounds*	*pounds*
4	Casein-cod liver oil	7	32	42.8	3.59	.21
6	Alfalfa hay	4	20	36.4	3.38	.01
3	Casein	12	56	43.8	3.37	1.24†
2	Cod liver oil	12	51	32.0	2.13	.02
1	Oat straw	7	28	34.5	2.11	.32
5	Alfalfa ash	10	39	29.2	1.79	
			Twins			
4	Casein-cod liver oil	4	26	40.6	1.93	.08
6	Alfalfa hay	8	70	38.8	1.85	.04
3	Casein	2	18	38.0	1.81	.44*
1	Oat straw	4	32	37.2	1.37	.15
2	Cod liver oil	2	24	36.5	1.22	.01
5	Alfalfa ash	1	12	30.5	1.21	

[1] Data taken during period of milk production observations.
* Significant difference.
† Highly significant difference.

casein lots (2 and 3) but was significantly higher in all three of these lots than in lots 1 (oat straw), 2 (cod liver oil) and 5 (alfalfa hay ash). These results indicate that the addition of casein and cod liver oil to the oat straw ration definitely improved its nutritive value insofar as milk production was concerned.

Since growth of the lambs was one of the main objectives of the experi-

ment, weekly weights were recorded over the seven-week period of milk production observations. The gains in weight of single and twin lambs were treated separately (table 3). The same conclusions result from analysis of gains of either singles or twins. Nonsignificant regression coefficients of gain per week on age were 0.0005 for singles and 0.001 for twin lambs, indicating that the rate of gain did not vary during the seven-week observation period. This removed the necessity of adjusting the lot mean gains per week to the average day of lactation. In general, the growth rate of the lambs correlates very well with the milk production, viz.; lambs from ewes with the highest milk production grew the fastest. The single lambs in the alfalfa hay, casein, and casein-cod liver oil lots all grew significantly faster than those in the oat straw, cod liver oil and alfalfa hay ash lots. There were no significant differences in growth between the lots within these two groupings.

In general, the results were the same with the growth of the twins. The twin lambs grew at a slower rate than the singles, but the differences between the faster and slower gaining groups was less. However, the division into two groups was the same as in the case of the singles; lots 4, 6, and 3 growing faster than lots 1, 2, and 5. The differences in gain between lambs in lots 4 and 6 and 1, 2, and 5 was highly significant and the difference between lots 3 and 2 and 5 was highly significant.

These results indicate that alfalfa hay contains a factor or factors required by breeding ewes for optimum milk production and growth of lambs. The protein from casein and vitamin A did not entirely supplement the oat straw ration. Experiments are underway in which various fractions of alfalfa are being fed in an attempt to determine, more exactly, the deficiencies of non-leguminous rations for sheep.

Summary

1. Studies were made on maintenance and milk production of ewes and growth of lambs fed rations containing non-leguminous roughage (oat straw) supplemented with protein, vitamin A and alfalfa hay ash. An unsupplemented oats straw ration and an alfalfa hay ration were fed as controls.

2. The results of this experiment based on milk production of the ewes and growth of lambs, indicate that the non-leguminous ration of corn, corn silage, and oat straw was deficient in protein and probably also in vitamin A.

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THE EFFECT OF FLUORINE IN ROCK PHOSPHATE IN THE NUTRITION OF FATTENING LAMBS[1]

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PREVIOUS studies by various investigators have shown that fluorine may be toxic when present in considerable quantities in the ration of animals. Most of this work has been done with man, swine, cattle, and rats, but very little work has been reported with sheep. It is important to know the safe levels for the ingestion of fluorine in different types of animals. The object of this work was to determine the fluorine tolerance of sheep when rock phosphate is used as the mineral supplement.

Some of the effects of fluorine in the diet may be summarized briefly. Excessive amounts of fluorine lead to poor growth and poor food consumption which is very important in domestic animals. Investigations have shown that the mottled appearance of human enamel in certain sections of the country is due to excessive amounts of fluorine in the drinking water (Cox, 1940), and similar results have been noted in feeding experiments (Pierce, 1938). High fluorine intake will tend to produce bones which are larger but weaker, have slightly greater ash content, and contain considerably more fluorine. Blood analyses show that the calcium, although still in the normal range, decreases to some extent.

The source of fluorine in the ration affects the level below which no detrimental effects are noticed in growth and food consumption (Evans and Phillips, 1939). The more soluble forms, sodium fluoride for example, inhibit growth and food consumption to an appreciably greater extent than some of the less soluble forms, such as calcium fluoride, cryolite and rock phosphate.

Kick and coworkers (1935) made a comprehensive study of the effect of fluorine on rats and swine. They found that the toxicity varied with the form in which fluorine was fed. When pig rations contained more than 0.029 per cent of fluorine as sodium fluoride or more than 0.033 per cent as rock phosphate a definite deleterious effect on teeth and bones was observed.

Goldemberg (1926) calls the effect on growth a "cretinisme fluorique," suggesting an endemic cretinism due to the effect upon the thyroid gland. There has been no experimental confirmation of this work in spite of several efforts. Recent studies have been made on the effect of fluorine on basal

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Some of the data presented are from a thesis to be submitted by J. D. Hatfield to the Graduate School of Purdue University in partial fulfillment of requirements for the degree of Doctor of Philosophy.

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metabolism (Phillips, 1935, 1936). These results have shown that fluorine alone does not lower the basal metabolic rate but that it enhances the toxicity of hyperthyroidism induced by feeding desiccated thyroid. When fed with desiccated thyroid it causes a greater rise in the basal metabolic rate than that induced by the desiccated thyroid alone. The same investigators worked with patients undergoing thyroidectomies and found no definite evidence that fluorine in any way plays a part in human hyperthyroidism (Evans and Phillips, 1938).

Materials and Methods

Twenty western lambs of approximately the same age and weight were divided into four groups of five each. The lambs were kept in an unheated barn and were bedded with straw on a concrete floor. The rations consisted of ground yellow corn, mineral supplement containing fluorine, and alfalfa hay. The mineral supplement consisted of rock phosphate containing 3.85 per cent fluorine and special steamed bone meal to balance the calcium and phosphorus intake of all the animals. The amount of rock phosphate in the ration was adjusted to give the following daily intakes of fluorine per sheep:

Lot I —No fluorine.
Lot II —1.5 mg. of fluorine per kilogram of body weight.
Lot III —3.0 mg. of fluorine per kilogram of body weight.
Lot IV —6.0 mg. of fluorine per kilogram of body weight.

Immediately it was found that the animals in Lot IV refused to eat the grain when the mineral was added. For this reason it was found necessary to introduce the minerals mechanically in gelatin capsules. Even then the grain consumption was markedly reduced in comparison with the other lots.

The animals were weighed every 10 days to adjust the daily intake of fluorine with the changing body weight. Blood samples were secured before slaughtering. The animals were slaughtered after being on the experimental rations for 170 days. The left humerus, the lower jaw with the teeth intact, and the thyroid gland were taken from each carcass. At the time of slaughtering the carcasses were examined for abnormalities.

Results

The results of growth, grain consumption, and blood analysis are given in table 1. It will be seen that the best gain was obtained in lot III followed by lots II, I and IV respectively. The grain consumption varied from lot to lot in the same manner. Upon calculating the grain consumed per pound of gain in body weight, it is found that lot III made the most rapid gains followed by lots II, I and IV, respectively. It appears that the levels of fluorine for lots II and III were not detrimental for fattening lambs as far as growth

TABLE 1.—THE EFFECT OF DIFFERENT AMOUNTS OF FLUORINE IN ROCK PHOSPHATE ON GROWTH, FEED CONSUMPTION AND BLOOD OF FATTENING LAMBS

Lot	No. of lambs	Growth and grain consumption				Blood analysis	
		Average initial weight	Average gain	Average grain consumption	Grain consumed per pound of gain	Ca mg./100 ml.	P mg./100 ml.
		lb.	lb.	lb.	lb.		
I	5	61	39.5	166.6	4.22	12.35	3.59
II	5	60	43.0	167.6	3.90	12.71	3.76
III	5	60	47.5	176.9	3.72	11.83	3.85
IV	5	61	31.0	136.7	4.41	10.99	3.96

and grain consumption are concerned. The fluorine consumption of lot IV (6.0 mg. daily per kilogram of body weight) resulted in a decrease of grain consumption and growth. The difference in consumption of grain per pound of gain in the different lots was not considered to be significant.

In table 1 is also listed the average values for the calcium and phosphorus in the blood of the different lots. There was a considerable decrease in the calcium content and a progressive increase in the phosphorus content in going from lot I to lot IV. These values are still within the normal range for sheep, but it is of interest to note that blood calcium decreases and blood phosphorus increases with increasing fluorine intake.

In table 2 are given the bone measurements and analyses of the left humeri of the slaughtered animals. The bones were weighed on an accurate balance, and the volume determined by displacement of sand to calculate the density. The lengths of the bones, minimum diameters and wall thicknesses were determined by use of calipers and a ruler. The breaking strength

TABLE 2.—THE EFFECT OF DIFFERENT AMOUNTS OF FLUORINE IN ROCK PHOSPHATE ON THE BONES OF FATTENING LAMBS
(Figures are averages for 5 animals in each lot)

Lot	Average weight	Average density	Average length	Average minimum diameter	Average wall thickness	Average breaking strength	Breaking strength per gm. weight average	Average ash	Average fluorine in fat free bone
	gms.	gms./ml.	inches	cms.	inches	lbs.	lbs./gm.	per cent	per cent
I	96.6	2.147	5.24	6.84	.136	933.8	9.668	64.04	.035
II	100.0	1.667	5.23	7.00	.145	959.0	9.655	64.62	.203
III	107.7	1.657	5.22	7.10	.140	1000.0	9.266	64.99	.280
IV	104.6	1.674	5.35	7.02	.134	857.2	8.143	64.84	.401

was determined by a special apparatus, and the breaking strength per gram of weight was calculated. The ash content was determined by burning the extracted bone for 24 hours in a muffle at 600° C. The fluorine content of the ash was determined by the Willard and Winter method, as modified by Reynolds and Hill (1939).

The total weight of the bone and the breaking strength were in the same order as the total gain in weight of the animals (table 1). The animals which gained most, Lot III, had the heaviest bones and highest breaking strength, but the breaking strength per gram of bone shows a marked decrease from lot I to IV. There was very little difference in this respect between lots I and II, somewhat greater difference between lots II and III, and a remarkable

TABLE 3.—THE EFFECT OF FLUORINE IN ROCK PHOSPHATE ON THE THYROID GLAND OF FATTENING LAMBS

Lot	Fresh wt. total	Dry wt. total	Iodine in dry gland	Total iodine in gland	Ash	Salt consumed	Iodine consumed	Total iodine ÷ iodine consumed
	gms.	*gms.*	*per cent*	*gms.*	*per cent*	*lbs.*	*gms.*	
I	60.06	13.0102	.009	.00117	6.27	5.0	.172	6.8×10^{-3}
II	34.88	8.4112	.037	.00311	5.03	5.0	.172	18.1×10^{-3}
III	20.41	5.6095	.071	.00398	3.56	6.0	.207	19.2×10^{-3}
IV	10.02	2.8858	.160	.00462	3.55	8.0	.275	16.8×10^{-3}

difference between lots III and IV. The breaking strength per gram of bone was inversely proportional to the amount of fluorine in the ration.

The percentage of ash increased perceptibly, although not absolutely regularly, as the fluorine was increased in the ration. The amount of fluorine in the bone increased by a very significant quantity as fluorine was increased in the ration. It may be that the decrease in breaking strength per gram of weight was due to the increase of fluorine in the bone.

One or two animals in lot IV showed a slight discoloration of the teeth, but the animals as a whole had excellent teeth. There was little perceptible difference in the teeth of the animals in the different lots.

The data on the thyroid glands represent the total for the five animals in each lot. It was noticed during the experiment that the water consumption increased from lots I to IV. At the end of the experiment it was found that there was a difference in the salt consumption of the different lots (table 3). Each lot had access to a salt block which was non-iodized but which contained 0.0076 per cent iodine.

The total weights of the five glands from each lot are given in table 3. There was a very marked decrease in the fresh weights and in the dry weights

as fluorine was increased in the ration. The percentage of iodine in the glands increased as fluorine was increased in the ration. There was found to be a progressive increase in total iodine in the glands from lot I to lot IV. From the amounts of salt consumed in the 170 day experimental period can be calculated the total iodine consumed in this period. Naturally this is a rough figure as the measurement of salt consumed occurred as an afterthought, and the figures are not too accurate. Also, the slight amount of iodine in the feed and minerals was not taken into consideration. However, there occurred an increase in the iodine consumption and an increase in iodine in the thyroid glands as fluorine was increased in the ration. The ratio of the total iodine in the gland to the iodine consumed is given in the last column. This ratio is practically the same for lots II, III, and IV and only one third as great for lot I. It would seem from this that the effect of fluorine upon iodine utilization is a constant even at different levels of fluorine. It may be that the iodine alone caused the decrease in the size of the gland. If this were the case there should be no difference between the sizes of lots I and II thyroids. Perhaps fluorine influences in some way the effect of the iodine, or perhaps there is an effect upon the thyrotropic principle in the pituitary gland. The experiments are being continued.

The fact that the calcium content of the blood was affected raises the question of a possible effect of fluorine on the parathyroid glands. However, no data were obtained on the parathyroid glands.

Summary and Conclusions

1. Rock phosphate containing 3.85 per cent fluorine was fed as a mineral supplement to fattening lambs in amounts furnishing 1.5, 3.0, and 6.0 milligrams of fluorine per kilogram of body weight per day.

2. Grain consumption was decreased and growth depressed in the lot receiving 6.0 milligrams of fluorine per kilogram of body weight per day.

3. As the fluorine intake was increased the breaking strength per gram of bone decreased proportionally, and the percentages of ash and fluorine in the bone increased.

4. The weight of the thyroid gland decreased as the amount of fluorine was increased in the ration.

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WOOL YIELDS IN THE SMALL SIDE-SAMPLE AS RELATED TO INDIVIDUAL WHOLE-FLEECE YIELDS IN FOUR BREED-GROUPS OF SHEEP

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Introduction

THE importance of estimating the yield of wool in the whole fleece from that in a small sample has been recognized generally by workers interested in sheep-improvement problems. Methods of sampling and yield determination have been extensively studied and described in detail by Buck (1940), Burns (1938, 1940*a*, 1940*b*), Hardy (1927, 1934), Spencer, Hardy, and Brandon (1928), and Wilson (1938). A study of the application of the techniques to selection problems was begun by Spencer and coworkers (1928). The study by Spencer and coworkers was done primarily on the Rambouillet breed where a close agreement was found between the side-sample and whole-fleece yields aside from the elements of grease and dirt that account for the general shrinkage in yield determination. The work of the other writers referred to above also places emphasis on the reliability of the small sample as representative of the whole-fleece yield, although these studies have not been made with respect to breed relationships.

It was in order, then, to apply the yield-sampling methods to several breed groups of sheep in order to determine whether breed-group differences exist, and if so, to develop an index for selection for each. Such a study is reported in this paper representing Rambouillet, Targhee, Corriedale, and Columbia sheep. Since questions concerning the relationships between yield and density index, fiber fineness, and weight of whole fleece are always pertinent to fleece yield studies, consideration is also given to these in this report.

Material and Methods

A study was made of 174 fleeces from yearling ewes at the Western Sheep Breeding Laboratory and United States Sheep Experiment Station, Dubois, Idaho, in 1938 and 1939. The fleeces were produced under intermountain range conditions at an altitude of 5,000 to 8,500 feet above sea

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level. Typical spring, summer, fall, and winter range areas were used for grazing, and feed lot care was provided during the extremely cold winter months. Of the fleeces used, 45 were Rambouillet, 40 Targhee, 44 Corriedale, and 45 Columbia. There were 154 fleeces from the 1938 clip and 20 from the 1939 clip. The average and variation of yields of the 20 fleeces and samples were practically identical with their respective groups of the 1938 season; therefore, no adjustments for annual differences were necessary.

The samples were taken midway between the scapula and hip-joint and between the backbone and belly on the middle of the right side. This area, cleared with an electric clipper (Shearmaster) was approximately 5.2 cm. wide and 11 cm. long, and yielded 25 to 35 gm. of wool. The wool samples were immediately placed in moisture-proof containers and the cleared area was measured for density determination. The procedure was similar to that described and illustrated by Hardy (1934) for taking yield and density samples. A sample consisting of a small lock of wool was taken adjacent to the cleared area for the determination of fineness (Hardy, 1935). Since the determinations of the density index, fiber fineness, and weight of whole fleece on these sheep were made and used as parts of other reports, these data were available for comparisons in this study on yields.

After being weighed, the wool in the small samples was opened by hand, and the whole fleeces were passed through a mechanical fleece opener, to remove loose dirt and vegetable matter. The scouring was done by the emulsion process, described in detail by Hardy (1927), Wilson (1938), Burns (1940a), and Buck (1940). The percentages of yield are based on bone-dry determinations of clean wool for both the small samples and whole fleeces. It should be noted that bone-dry determinations free the wool from moisture variation, giving a controlled basis for analytical comparisons. Yields of wool according to the commercial standard are reported on the basis of the clean wool containing 12 per cent moisture (Buck and La Compte, 1941). In order to convert bone-dry clean yields to standard commercial yields the bone-dry yields should be divided by 0.88. The percentage yields of the small samples were determined at the Western Sheep Breeding Laboratory of the Bureau of Animal Industry, and the whole fleece yields were determined by the Agricultural Marketing Service in conjunction with studies on commercial yields of wool. The statistical procedures used for analysis of the data followed those given by Snedecor (1937) for regression, covariance, and multiple covariance.

In order to establish an index for prediction the first consideration is which of the two regressions inherent in the paired data becomes the logical one to use. The usual procedure of prediction is from the independent variable X to the dependent variable Y, making use of the $b_{x \cdot y}$ coefficient with

its corresponding a. However, in the practical application of these data it is desired to predict the whole fleece value X in terms of the sample Y. In this case, then, the $b_{y \cdot x}$ coefficient should be used (Eisenhart, 1939). The relationship is expressed by the equation $\frac{Y-a}{b_{y \cdot x}} = X \pm s_{y \cdot x}$, in which Y is the observed value of the sample, the constants a and $b_{y \cdot x}$ are calculated from the data, X is the predicted value and $s_{y \cdot x}$ is the standard error of estimate calculated from the data.

Data and Discussion

The correlations between the small sample and whole fleece yields (table 1) are of interest because they describe the relationship between these

TABLE 1.—YIELDS IN WHOLE FLEECES AND SMALL SAMPLES FOR THE FOUR BREED-GROUPS, AVERAGES, CORRELATIONS, AND REGRESSION COEFFICIENTS

Breed groups	Fleeces and samples	Average yield[1]			Regression coefficients[2]		Correlation r
		Whole fleece	Small sample	Adjusted means	a	$b_{y \cdot x}$ with standard error	
	number	*per cent*	*per cent*	*per cent*			
Rambouillet	45	35.6	35.6	38.3	−2.86	1.11±.146	.76
Targhee	40	37.0	38.3	39.6	−1.62	1.08±.174	.71
Corriedale	44	39.6	42.0	40.3	−0.84	1.18±.117	.84
Columbia	45	40.3	44.2	41.9	+0.73	1.01±.261	.88
Average or total	174	38.149	40.052	—	—	1.08±.062	.80

[1] For those accustomed to thinking in terms of yield on a commercial basis, the values on the bone-dry basis may seem low for the breed groups. The bone-dry values divided by .88 will give the approximate commercial yield; for example, for the Rambouillet breed the approximate average commercial yield would be $\frac{35.6}{.88} = 40.5$, or 12 per cent higher than the bone-dry determination.

[2] a and b are the coefficients necessary to the expression of any linear regression.

values within the breed groups, and throughout the data taken in total. These correlation coefficients do not enter directly into the applied prediction, but they do serve to emphasize the consistent relationships inherent in the data from which the regression or prediction formula is developed. The correlation of .76 and the identical averages of the yields of the small samples and whole fleeces of the Rambouillet group are of interest in comparison with observations made on Rambouillets by Spencer *et al.* (1928) where the correlation was .75 and the averages of the yields of side samples and whole fleeces were practically identical.

The averages for the percentage yields of the whole fleeces and small samples (table 1) show that a progressive difference exists between Rambouillet, Targhee, Corriedale, and Columbia groups. That is, a given small sample yield indicates a higher total yield in the Rambouillet than in the Columbia. This finding suggests that breed-group differences exist, and lead to the covariance analysis (table 2) which provides the test for significance of breed-group differences. The test proceeds on the basis of adjusted breed-group means compared to a common mean derived from all the data. These values are entered in table 1 since they function directly in the expression of the regression constants. The completed test given by the F ratio in

TABLE 2.—ANALYSIS OF COVARIANCE AND TEST OF SIGNIFICANCE OF ADJUSTED BREED-GROUP MEANS

Source of variation	Degrees of freedom	Sums of squares and products			Adjusted sum of squares	Degrees of freedom	Mean square
		Sx^2	Sxy	Sy^2			
Between breeds	3	641.57	1108.29	1948.22	—	—	—
Within breeds	170	1820.54	1965.37	3306.31	1184.59	169	7.009
Total	173	2462.11	3073.66	5254.53	1417.42	172	—
For test of significance of adjusted means[1]					232.83	3	77.61

[1] An F ratio of 3.91 or greater is highly significant $\frac{77.61}{7.009} = F = 11.07$.

conjunction with table 2 indicates that the breed groups differ significantly in yield. Therefore, prediction of yields must be confined to each group in terms of their respective a values given in table 1. The a value fixes the average or level for each group that enters into the prediction equation, and is derived from the equation, $a = \overline{Y} - b_{y \cdot x}\overline{X}$, where $b_{y \cdot x} = 1.08$ and the $\overline{Y}$ and $\overline{X}$ are the observed breed means of the small sample and whole fleece yields, respectively.

The regression constants, $b_{y \cdot x}$, and their respective standard errors given in table 1 were calculated separately for each group and the values for the total were derived from the covariance analysis. The striking feature of these values ($b_{y \cdot x}$) is their consistent approximation of 1 and their significantly low sampling errors as expressed by their respective standard errors. All these regressions are slightly greater than unity. However, when these were tested against a theoretical regression of unity, no single coefficient differed significantly from a regression of $\beta = 1$. A test for homogeneity of the 4 group regressions was also made from the covariance analysis. This test indicated that these are homogeneous when breed-group differences are held constant.

This and the above tests indicate that the rates of change in the regressions as expressed by the $b_{y \cdot x}$ coefficients are essentially similar in the 4 groups. The optimum, or average within group, estimate of $b_{y \cdot x} = 1.08 \pm .062$, derived from the covariance analysis, can, therefore, be used for any of these groups in expressing the values for the prediction equation.

The standard error of estimate $s_{y \cdot x}$ for any predicted X will be one figure derived from the within group values, table 2, just as the $b_{y \cdot x} = 1.08$ applies to the prediction in any of the 4 groups. This $s_{y \cdot x} = \pm 1.18$ per cent, and is understood to describe approximately $\frac{2}{3}$ of the range within which similarly predicted X values will fall. The usual method of describing the $s_{y \cdot x}$ is to compare it with its related standard deviation to show the percentage reduction in estimation errors. The standard deviation here is the s derived from the within-breed mean square, table 2, and equals 2.65. The value 1.18 is 44.53 per cent of 2.65. Therefore, the prediction of the whole fleece yields from the small sample yields is 44.53 per cent more precise than if the regression values were not known.

The most striking feature brought out by the analysis of the data is the significantly different levels in yield between the four groups. It was thought that these group differences might be explained by such factors as fineness of fiber, density index, or weight of whole fleece. Data on these factors were available on 38 Rambouillet, 39 Corriedale, and 40 Columbia fleeces. However, a multiple covariance analysis showed that the group differences could not be accounted for in terms of these variables.

The consistent behavior of the regression values for the separate groups and the average within-group regression indicates that the yield determination techniques must have been uniformly applied throughout. The multiple covariance analysis also indicates that these techniques have operated independently of fineness of fiber, density index, and weight of whole fleece. The uniformity of regression rate along with the standard error of estimate emphasizes the confidence that can be placed in the prediction of whole fleece values from side sample yields when group differences are taken into account.

Application of Results

The application of the prediction index is in the form of the following equation, $\frac{Y - a}{b_{y \cdot x}} = X$, where Y = per cent clean yield of small sample, the a value will be that for the breed group from which the sample is taken (table 1), and $b_{y \cdot x} = 1.08$. Thus, a predicted value of whole fleece yield, X, from an observed small sample yield of 40 per cent from a Corriedale would

be calculated as follows: $\frac{40-(-.84)}{1.08}=37.8$.

The standard error of estimate, ± 1.18, applies to any predicted values in the four breed groups, so the value could be expressed as 37.8 ± 1.18.

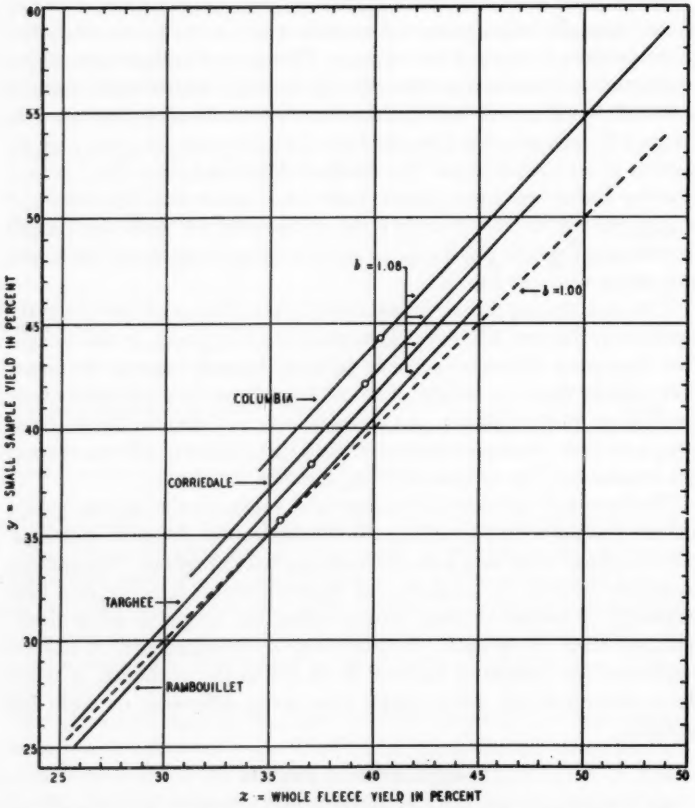

Figure 1. Regression relationship of yields of small samples on whole fleece yields in four breeds of sheep with $b_{y \cdot x}=1.08$, the breed means indicated by o, the regression lines giving the range of each breed, and the slope of a theoretical regression, $b=1.00$, given by the broken line.

The actual weight of clean wool is obtained by multiplying the weight of grease wool by the X value. Thus, if the Corriedale ewe in the above ex-

ample produced 10 pounds (4.536 kilograms) of grease wool, the yield of clean wool would be 3.78 pounds (1.73 kilograms).

The relationship of the four groups in terms of the regression rate, or slope of line, as expressed by $b_{y \cdot x}$, and the different group levels as expressed by the a coefficients are illustrated in figure 1. This figure also provides a scale by which estimates of whole fleece yields can be read directly from an observed sample yield for any of the 4 breeds without use of the prediction formula. Knowing the small sample yield in per cent, one simply reads the whole fleece yield from the chart at the point where the small sample yield intercepts the regression line for the breed involved.

It should be remembered that this paper is based on yearling sheep maintained under intermountain range conditions near Dubois, Idaho, and the facts set forth herein are for the purpose of illustrating a method of estimating clean yields of fleeces with small samples obtained from experimental sheep. The use of a regression chart such as is illustrated is regarded as useful when it appears that the need for accuracy is great enough to justify its development. It would be desirable to use this regression chart with caution and to watch for opportunity to conduct similar investigations with sheep of other breeds, ages, and environments.

Summary

The percentage yields of small side samples from 45 Rambouillet, 40 Targhee, 44 Corriedale, and 45 Columbia yearling ewes were compared to the percentage yields of the whole fleeces for the purpose of establishing a yield prediction index for these breed groups. Highly significant group differences were found in the side sample yields. Fineness of fiber, density index, or weight of whole fleece did not account for the group differences.

The regression rate was highly uniform in sampling error and nearly equivalent to $\beta = 1$, when the groups were considered separately or in combination. The average within-group regression rate was $1.08 \pm .062$, and was found to be applicable to all 4 groups. The average within-group standard error of estimate was ± 1.18 per cent which lowers the error of estimation by 44.53 per cent from estimation based on the sample alone. The complete prediction index is expressed in the form of a regression equation in which the group level is fixed by the a value of the respective breed group.

On the basis of the results obtained, the whole fleece yield, X, is calculated from the small sample yield, Y, using the formula, $\frac{Y-a}{b_{y \cdot x}} = X$. The values for a are -2.86, -1.62, -0.84, and $+0.73$, respectively, for the Rambouillet, Targhee, Corriedale, and Columbia groups. The value of $b_{y \cdot x}$ is 1.08 for all groups.

A graph illustrates the relationship between the breed averages and gives the respective breed regressions from which whole fleece values can be read directly when small sample yields are known.

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CORRECTION OF HEAT PRODUCTION FOR CHANGES IN LIVE WEIGHT OF CATTLE IN BALANCE EXPERIMENTS[1]

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THIS paper presents a method of correction of the heat production of cattle on account of change in live weight in the course of energy metabolism experiments, this correction being based on the balances of carbon and nitrogen coincident with the changes in live weight. It is applicable in a series of feeding periods in which analyses of feed and excreta are made and does not concern animals in the fasting state. It deals primarily with the matter of accepting with confidence the results of the balances of nitrogen and carbon rather than live weight changes as indicated by scales.

The usual methods of correction of the heat production of animals to correspond to a uniform live weight depend on the conception that the basal metabolism is proportional to the surface area, or to some power of the body weight. The accepted relationship existing between surface area and basal metabolism is not questioned. The live weight of an animal, however, includes the contents of the alimentary tract; and we have found by actual measurement in slaughter experiments that the weight of this item may differ as much as 16 kg. between steers treated identically and receiving a maintenance ration. Likewise, we have observed a difference of 19 kg. in the alimentary fill of two similarly treated steers at the end of four days of fast. These differences in the alimentary fill of identically treated animals were due to water. Furthermore, we have noted several instances of apparently consistent series of daily live weights of steers at the beginning and the end of 28-day experimental periods which indicated submaintenance planes of nutrition, while our concurrent balances of nitrogen and carbon showed that the steers were storing appreciable amounts of protein and fat.

This important finding may be illustrated by the following data taken from calorimetric experiments as yet unpublished. In two periods to be compared the live weights were taken as the average of the last 5 days of each period, each weight being obtained just before the steer was watered and at exactly the same time of day. The feed intake was kept absolutely constant in each period, the first one being at about twice the maintenance level and the second period somewhat above the maintenance level. The five weights

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in period one were 447.8, 455.0, 452.4, 454.4 and 457.4 kg. In the second period they were 436.4, 441.8, 437.2, 435.2 and 441.8. The averages for the two periods were 453.4 and 438.5 kg., respectively.

During the interval of 28 days which occurred between these two series of observations the steer gained daily 11.4 gms. of protein and 281.4 gms. of fat as shown by painstaking calorimetric measurements and chemical analysis of feed and excreta. In the 28 days this amounted to $28\times11.4\times5+28\times281.4$ or about 9.5 kg. of body tissue including the fat. (The weight of fresh body muscle, according to DuBois (1936, p. 25), is considered to be about 5 times its weight of dry protein.) Thus, the true live weight should be taken as 9.5 kg. greater for period 2 than for period 1, instead of 14.9 kg. less, as would be the case if the live weights of period 2 were accepted at face value. The error would be 24.4 kg. which signifies some 317 Cal. per day as indicated below. The series of live weights obtained during period 1 are not, of course, to be considered as being in any way more significant than those of period 2. The average live weight as obtained in period 1 merely serves as a starting point, any subsequent change in live weight being computed on a critical basis.

Thus, a better basis than ordinary live weight measurements for obtaining the change in actual body tissue and in surface area (or $W^{.73}$) during a feeding period is available in the balances of carbon and nitrogen (fat and protein) which afford a much more significant basis than do live weights for judgment as to the gain or loss of body substance during a period of constant feed intake. In fact, as a basis for correction of the heat production, we may consider only the nitrogen balance if we agree with DuBois (1936, p. 255) that there is little or no continuing metabolism in body fat.

If, however, body fat is to be considered as active metabolic tissue and equivalent to body protein in its effect on basal metabolism, then the significant gain in live weight would be the sum of these two amounts over a period of time involved. According to DuBois (1936, p. 25) the weight of muscle, including its moisture content and smaller amounts of other components, is about 5 times the weight of its protein; and analyses of the entire bodies of cattle by Trowbridge, Moulton, and Haigh (1922), reported by Armsby and Moulton (1925), confirm this value, the average protein content of 12 thin bodies being found to be 19.55 per cent on the empty weight basis (table 12, p. 40, 41). Thin animals were chosen in order to obtain as closely as possible the ratio of fat-free body tissue to body protein.

Although the work reported by Bruhn and Keller (1941) indicates that fat deposited in the body produces an increase in metabolism equal to that resulting from normal growth, it is also true that in so far as the process of fattening tends to replace water with fat in the muscle of the body there

would be partial compensation of any error involved in computing the weight of muscle from the nitrogen balance. However, Bodansky (1938, p. 348) points out that not all the fat in the body has the same physiological significance. The amount of fat in certain organs remains constant regardless of diet, whereas the storage of fat in muscle tissue and fat depots varies directly with the plane of nutrition. The lowest plane of nutrition properly used in obtaining production values is that of energy equilibrium, so that no severe depletion of fat deposits would exist in any case. For those workers who have a measure of the protein stored (nitrogen balance) but who do not have a measure of the fat stored (carbon balance) it seems justifiable to consider that the fat gained is primarily inert and is without appreciable effect on the basal metabolism. In any case the change in the amount of body fat during an experimental period of 28 days is very small as compared to the change in live weight which may occur due to a change in alimentary fill. The point to be stressed is not that this procedure results in perfection but rather that it avoids using values which are without definite significance and are subject to gross error.

Although a definite relationship between body weight and heat production of cattle must be used as a standard, it is the change in weight of body tissue during a feeding period which must be known, rather than the actual live weight of the animal. A correction for standard use was computed from the average live weight (432 kg.) and the average daily heat production (7681 Cal.), these being average values obtained from four fasting steers in a series of unusually consistent direct calorimetric studies at planes of nutrition ranging from fast to about three times the maintenance requirement by Forbes *et al.* (1928) (1930).

The application of the correction does not necessitate the determination of the fasting heat production, but may be used to correct the heat production of one feeding period to the basis of another such period. In accord with the observed heat production of fast, and in line with the idea that the basal metabolism varies directly with the 0.73 power of the live weight, we may say that $(432)^{.73}:(433)^{.73}::7681:X$. $X=7694$. Thus, a gain of 1 kg. in body tissue signifies an increase in heat production of 13 Cal. The correction for a gain of 25 Kg. based on the .73 power of the live weight signifies an increase of 322 Cal. Thus, over such an interval of change in live weight, the correction in heat production may be considered for practical purposes to be 13 Cal. per kg. change in live weight. If the computation is based on the $\frac{2}{3}$ power of the live weight, the correction becomes 12 Cal. per kg.

The absolute value of the heat production of fast, taken as a standard, will affect this value only slightly. For instance, if the heat production of the

fasting animal whose weight is 432 kg. is taken as 7181 Cal. instead of 7681 Cal. the correction for a gain in body weight of 10 kg. would be 121 Cal. instead of 130 Cal. The correction is applied to values ranging from 9000 Cal. upward. A possible error of 9 Cal., therefore, is not a serious matter. Furthermore, the correction would be applied to all animals, so that any such small inaccuracy would not be existent in a comparative sense.

A detailed study of the effect of the use of this correction on the curve of heat production of cattle in relation to food intake, as determined by Forbes *et al.* (1928) (1930), has been made. In the first series, done in 1928, the original corrections were not extensive, and the new basis of correction changes the heat productions by less than .4 per cent on an average, the greatest single change being a little less than 1 per cent. The curve of heat production as published, therefore, remains without significant change. In the second series much larger corrections were involved and the average change on the new basis amounts to 2.3 per cent of the heat production. The greatest change is 4.0 per cent, the next two being 3.8 per cent and 3.0 per cent. These three are at the highest planes of nutrition (two and one half or three times maintenance) and all are positive. Thus, the slight change would be to increase the slope of the curve in this region. The curves for the four animals would become even more closely similar than as originally published.

The method advocated for correction of the heat production of cattle, in accord with a measured gain of body tissue, should be much more significant than one based on changes in live weight, which is without critical scientific value.

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EFFECT OF DILUTERS AND STORAGE UPON FECUNDITY[1] OF BOVINE SEMEN

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THE resistance of spermatozoa to dilution with numerous isotonic solutions is well established, and the use of such solutions to increase the volume of the ejaculate, so that it can be utilized to inseminate a greater number of females, has been generally approved. Less is known, however, about the effect of semen diluters on the fertilizing capacity of spermatozoa. Obviously a prolongation of life of spermatozoa *in vitro* and the retention of their fecundity would increase the efficient use of a given semen sample. Of the various substances which have been reported to give an increased life duration of spermatozoa, lecithin has received the greatest attention recently. The addition of lecithin to a glucose-phosphate solution was found by Milovanov and Selivanova (1932) to prolong the life and retain the motility of spermatozoa. The origin of the lecithin had no significance. This effect upon spermatozoon motility of lecithin or phospholipids obtained from egg yolk or other sources and/or egg yolk itself has been verified by Phillips and Lardy (1940), and Lardy and Phillips (1941). The storing of semen in a buffered solution of egg yolk was reported by Phillips and Lardy (1940) and Willett *et al.* (1940) to have greatly enhanced the fecundity of the spermatozoa. On the other hand it was found by Underbjerg and Davis (1940) that stored semen samples which showed prolonged maintenance of spermatozoon motility in the egg yolk buffer pabulum or in modifications thereof gave conception percentages that differed little from undiluted stored controls.

In a search for other diluters it has been found by Militzer (1941) that autoclaved whole milk or a substance or substances present in milk prolonged the life and maintained motility of spermatozoa. Since milk is a biological fluid and has a pH nearly that of the egg yolk buffer it was thought that the former might have properties that were similar to the latter. In addition the autoclaved milk had the advantage of being a sterile solution.

Materials and Methods

Autoclaved whole milk and the egg yolk buffer solution were used as

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diluters for comparative studies. Fecundity studies were carried out in three herds[3] and included cows of the Holstein, Jersey, Guernsey, and Ayrshire breeds. The bulls used were of proven fertility and semen samples were obtained by means of an artificial vagina. The motility of each sample was determined at time of collection and prior to each insemination as described previously by Davis *et al.* (1940). The stored samples were held at a temperature of 4° to 7° C.[4] The design of the experiment, the treatments accorded the semen, the diluters used and the dilutions, a summary of the motility of the samples and age of the samples are shown in table 1. Whenever possible cows were inseminated in the various groups from a single ejaculate which had received the various treatments. Diluted semen corresponding to at least one cc. of undiluted semen was used for each insemination. The semen was deposited into the os cervix with a glass syringe attached to a glass or stainless steel inseminating tube. The conceptions reported mean that either calves have been dropped or the fetuses have been located by manual palpation per rectum.

Results and Discussion

Table 2 presents the results of inseminations employing the semen samples that received the various treatments which were shown in table 1. Since the significance of the differences in the conception percentages between the eight groups of cows was not readily recognizable a statistical treatment of the data was made. Chi-square values according to the method used by Snedecor (1940) were obtained on the conception percentages shown in table 2 using the control groups I and V as bases. The sum of the chi-square values for II, III, and IV, using 63.5 per cent as a base shows that $\chi^2 = 9.9942$. For 3 df, $P = .03$, which indicates that the treatments accorded the fresh semen tended to lower the conception percentage. With one degree of freedom the individual value of χ^2 of II was not significant; the values of χ^2 of III and IV, however, indicated a trend towards a significant difference. Thus the treatments with the stored egg yolk buffer and the autoclaved milk indicated a tendency to lower the fecundity. Using 36.7 per cent as a base for the stored groups the sum of the chi-square values for VI, VII, and VIII shows that $\chi^2 = 6.0546$. For 3 df, $P = .13$, which indicates that the treatments did not cause a significant departure from the conception percentage of the untreated stored control group.

[3] The University of Nebraska; State Reformatory, Lincoln; and the Purina Mills Experimental Farm, Gray Summit, Missouri.

[4] Some samples of undiluted semen and samples of semen diluted with autoclaved milk were shipped via R. R. Express from the University of Nebraska to the Purina Mills Experimental Farm. The semen vials were wrapped in paper and packed with cotton in a thermos bottle. An ice cube held in a thin rubber bag was placed in the bottom and at the top of the thermos bottle. The thermos bottle was wrapped with cotton and placed in two double padded corrugated paper boxes.

TABLE 1.—DESIGN OF THE EXPERIMENT
SUMMARY OF MOTILITY AND AGE OF SEMEN SAMPLES USED

Treatment of semen at time of collection	Fresh semen			Stored semen			
	Group of cows	Average motility of spermatozoa when used	Average age of semen when used	Group of cows	Average motility of spermatozoa when used	Average age of semen when used	
						All samples	Samples which caused conception
None	I	*per cent* 90	*hours* 1	V	*per cent* 54	*hours* 36 (24–123[1])	*hours* 33 (24–99)
Diluted 1:4 with fresh E.Y.B.[2]	II	90	1	VI	77	61 (24–177)	39 (24–123)
Diluted 1:4 with stored E.Y.B.[3]	III	90	1	VII	74	54 (24–144)	34 (24–72)
Diluted 1:1 with autoclaved milk[4]	IV	90	1	VIII	68	39 (24–120)	44 (24–93)

[1] Numbers in parentheses denote the range in age of the semen samples used for insemination.

[2] E.Y.B. Egg yolk phosphate buffer prepared as recommended by Phillips and Lardy (1940).

[3] Egg yolk phosphate buffer prepared as above but held in storage for an average length of 160 hours before use for dilution.

[4] The milk was collected in a sterile flask and autoclaved for twenty minutes at a temperature of 120° C.

The sum of the chi-square values of V, VI, VII, and VIII using 63.5 per cent as base shows that $\chi^2 = 122.9764$. For df, $P = < .01$, which is highly significant and demonstrates that during storage there is a marked decrease in the conception percentage despite the retention of motility of spermatozoa.

Homogeneity is assumed for the semen samples employed in the various treatments and for the cows used for inseminations in that the semen and the cows used in the treated groups had an equal chance with those in the untreated control groups. Based on the assumption that the control groups represented a reliable expectation in fecundity of undiluted semen it has been shown that the treatments had no significant effect on the variation in the conception percentages obtained. The variation in these conception percentages, therefore, must be due to factors other than treatment and motility. It will be noted from table 1, that the stored treated groups showed the highest percentage of motile spermatozoa on the average; yet, the conception

TABLE 2. EFFECT OF DILUTERS AND STORAGE UPON FECUNDITY OF BOVINE SEMEN

Treatment of semen at time of collection	Fecundity of fresh semen					Fecundity of stored semen				
	Group	Number of cows			Conception percentage	Group	Number of cows			Conception percentage
		Inseminated	Conceived	Did not conceive			Inseminated	Conceived	Did not conceive	
None	I[1]	*number* 575	*number* 365	*number* 210	63.5	V	*number* 106	*number* 39	*number* 67	36.7
Diluted 1:4 with fresh E.Y.B.	II	22	12	10	54.5	VI	112	29	83	25.9
Diluted 1:4 with stored E.Y.B.	III	21	9	12	42.9	VII	26	10	16	38.5
Diluted 1:1 with autoclaved milk	IV	43	20	23	46.5	VIII	26	8	18	30.8
SUMMARY OF ALL GROUPS										
I–IV incl.		661	406	255	61.4	V–VIII incl.	270	86	184	31.9
I–VIII incl.		931	492	439	52.8					

[1] Roman numerals denote the various groups of cows which were inseminated with semen that had been treated as outlined in table 1.

percentages (cf. table 2) were not significantly different from the control group which had a lower percentage of motile spermatozoa. The age of the stored samples used for inseminations varied markedly, while the age of the samples that caused conception did not vary greatly between the groups. Occasionally, however, aged semen samples caused conceptions, but these occurred in the untreated as well as the treated groups. It is not likely, therefore, that the age itself of the samples within the limits herein reported could have been the factor responsible for the decreased fecundity of the stored semen specimens. Logically other unknown factors are in operation which cause loss of the fertilizing capacity of spermatozoa despite the retention of a high degree of motility.

Apposite to this supposition is the report by Voloskova (1938) of the presence of lecithin in equine spermatozoa and in the sperm plasma. He found that the sexual activity affected the lipoid level of the semen. Following intensive activity this level decreased and a three to six days' interval was required to restore it. The lecithin content also affected the activity and survival of spermatozoa. Further it has been suggested by Zeller (1941) and Zeller and Joel (1941) that the motility of human spermatozoa is dependent upon two enzyme systems which normally exert their influence simulta-

neously; however, either one was able to maintain motility alone if one of the two systems was blocked. The two enzymes concerned, a diamine-oxydase and a monoamine-oxydase, were found mainly in the seminal fluid. It is thus probable that the retention and release of kinetic energy of spermatozoa is dependent upon an equilibrium of extra- and intra-cellular units. It is possible that these units play an equally important role in the fertilization process. When this equilibrium is out of order, however, despite the retention of motility a reduction in fecundity takes place.

It is interesting to note that if the data of table 2 are summarized the total conception percentage for the four stored groups is markedly lower than that for the four unstored groups; but the conception percentage obtained for all inseminations in the eight groups is rather good. Thus when the age of the semen samples and the control groups are disregarded the illusion may be created that treatment and dilution appear to be beneficial.

Summary

Fresh semen samples were treated and used for inseminations as follows: I, no treatment (control); II, diluted with fresh egg yolk buffer; III, diluted with stored egg yolk buffer; IV, diluted with autoclaved milk. Other samples V, VI, VII, and VIII receiving the same series of treatments respectively were stored prior to insemination. A total of 931 cows were inseminated in these eight groups of which 492 conceived. Conception percentages in the various groups were as follows: I, 63.5; II, 54.5; III, 42.9; IV, 46.5; V, 36.7; VI, 25.9; VII, 38.5; VIII, 30.8.

A test of significance employing chi-square values indicated that the treatments accorded the fresh and stored semen in the various groups did not cause a significant lowering of the conception percentages from the controls. Conversely the stored groups as a whole showed a highly significant departure from the conception percentage in the fresh control group despite the retention of a high degree of motility of the spermatozoa.

It is concluded that the treatments had little beneficial effect on fecundity, but that during storage other factors are in operation which cause loss of the fertilizing capacity of bovine spermatozoa regardless of retention of motility.

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TWINNING IN HORSES[1]

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MULTIPLE births are the rule in swine and occur frequently in certain other farm animals. Twins and triplets are frequently produced by sheep and goats and quadruplets are not uncommon in the latter species. In uniparous species such as cattle and horses, multiple births are much less frequent, however. Several workers have made studies of the occurrence of multiple births in cattle but little work has been done with horses. Horses are most similar to cattle in the frequency of multiple births of any of the farm animals. Therefore literature dealing with horses and cattle is reviewed briefly.

Robertson (1917) describes a mare that produced three sets of twins in nine foalings. In two cases twin mules were produced and in the third the twins were a female mule and a male horse colt. One of the single mare offspring of this mare had produced twins and a half-sister of the mare also had a set of twins. Wriedt (1928) found no indication that the tendency to produce twins was inherited in horses. Andrews and McKenzie (1941) found twin ovulations occurring in mares in 3.8 per cent of the cases examined.

Gowen (1918) found twins occurring in one out of 125 cases in cattle. White (1924), in a study of 1300 births in cattle, reports that twins occurred about once in 76 times. Lush (1925) found 16 pairs of twins in 181 births in Holstein cattle, or a ratio of 1 multiple birth to 11.31 single births.

Jones and Rouse (1928) in a study of beef cattle state that the number of twins increases with the age of the cow. Among 12- and 13-year-old cows, they found 6.92 cases of twins per 1000 births, or about one in 144.

In a study of 940 calvings, Lamb (1935) found 29 cases of multiple birth, a ratio of one multiple birth to 32.41 single births or 3.085 per cent. The ratio in the different breeds was as follows: Holsteins—1:23.3, Jerseys—1:58.33, Guernseys—1:82.5, Ayrshires—1:22.67, and Brown Swiss—no twins or triplets.

Considerable variation exists in findings of various workers, with a range from no multiple births to one multiple birth for 11.31 single births in cattle.

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[2] The authors acknowledge the assistance of Professor H. R. Hunt.

Material Used in Study

Since the data available in the literature on the occurrence of multiple births in horses are very limited, the records of the draft stud at the Michigan State College were examined to obtain such data. Two breeds, Belgian and Percheron, are maintained and records were available for 403 parturitions from 102 purebred mares.

The herd has been developed largely from two outstanding females. A Belgian mare, Pervenche 9030, was purchased in 1922. She has an outstanding record in the show-ring and as a brood mare. Nine of her female offspring or their descendants are now in the herd. A Percheron mare, Coreen 117580, was foaled in 1914 and purchased in 1923. In 25 years she produced 20 foals. Of this number 16 were strong, living foals. One pair of twins and one single were aborted, and one foal born in her last year died shortly after birth. The present Percheron herd is composed largely of Coreen's offspring and their descendants.

The stallions which have been used most extensively in the Belgian herd are Range Line Phoenix 12808, Preston Wolfe 14607 and Ginger 18711, and in the Percheron herd, Treviso 144394, Sir Laet 190277, Oak Forest Echo 183540 and Mediator 209523.

Data and Discussion

The data on the occurrence of multiple births are summarized in table 1, along with certain other related data.

TABLE 1.—SUMMARY OF FOALING DATA

Item studied	Breed		Total
	Belgian	Percheron	
Mares foaling (number)	46	56	102
Parturitions (number)	174	229	403
Male foals (number)	90	113	203
Female foals (number)	87	119	206
Pairs of twins (number)	3	10	13
Ratio of twin to single births	1:58	1:22.9	1:31
Twin births (per cent)	1.72	4.37	3.23

A total of 13 pairs of twins were recorded, 3 of these being in the Belgian and 10 in the Percheron herd.

The data seem to indicate a higher proportion of multiple births in the Percheron than in the Belgian group but the proportions of twins in the two groups were not found to be significantly different ($P = .10$), using the chi-square test.

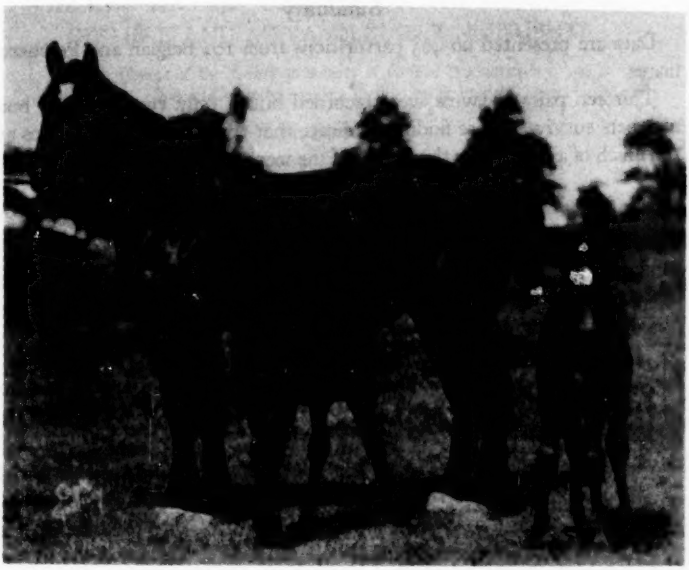

Figure 1. One of two complete pairs of twins raised at Michigan State College.

Seven of the 13 pairs of twins were aborted early in the gestation period. There were only 9 abortions out of 390 single births which is significantly less abortions than in the case of twin births. In two cases of twin births, only one individual was alive at birth and in two cases both were dead or died soon after birth. In the two other cases normal twin fillies were foaled. One of the normal pairs of twins is shown in figure 1. The dam of these foals suffered much from this difficult parturition and its after effects. While these data may not be representative, they seem to indicate that the uterus of the mare is not constructed to care for two embryos at one time. Multiple pregnancies in horses, might, therefore, be considered abnormal since death of one or both twins so frequently results. This conclusion is in accord with the opinion of Williams (1929), who believes that the burden of producing one normal young per gestation is all that should be expected of such animals as the mare and cow.

The sex ratio for all parturitions was near equality, since 203 males and 206 females were produced. The sex of several of the twins which were aborted was not recorded, hence these figures do not check exactly with the number of parturitions.

Summary

Data are presented on 403 parturitions from 102 Belgian and Percheron mares.

Thirteen pairs of twins were recorded but in only two pairs did both members survive. These findings indicate that two developing embryos are too much of a burden for the uterus of the mare in most cases.

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THE EVALUATION OF FEEDS ON THE BASIS OF DIGESTIBLE AND METABOLIZABLE NUTRIENTS

Report of the American Society of Animal Production's Special Committee on the Evaluation of Feeds[1]

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THE initial wastages that the nutrients of rations undergo in the animal body occur in the alimentary tract. For farm animals subsisting on practical farm rations these wastages are considerable, especially for ruminants and horses that consume bulky rations containing high proportions of roughages.

There are marked differences among feeds and rations in the extent to which nutritive material is lost in the digestive process, and among different species of animals differences in digestive efficiency exist. And finally among animals of the same species the completeness with which nutrients are utilized in digestion exhibits considerable variation. In a compilation of the results of digestion experiments with ruminants on American farm feeds Fraps (1925) concludes:

"It is an even chance that differences in digestion of individual animals may cause variations of about 3 per cent of the productive value with corn, wheat, and a few similar feeds, about 6 per cent with the usual run of feeds, and about 14 per cent with some low-grade feeds." However, Ringen (1940) believes that individual variation is often exaggerated by technical errors in the determinations of digestion coefficients.

The extent of the digestive wastage of nutritive material in farm feeds and rations contributes greatly to the importance of its accurate measurement for any system of the nutritive evalutation of feeds, while the variation to which the wastage is subjected indicates that reliable average measurements can result only from the use of many different samples of feed and many experimental animals. The problem is a statistical one, requiring for its solution, not only a considerable accumulation of experimental data, but also the use of technical statistical methods of assessing reliability of average results and of establishing and interpreting relationships.

However, the ordinary metabolism and digestion experiment does not measure all of the wastage of the nutritive material in the alimentary tract, since some of this wastage is in the gaseous form, representing the activity

[1] The members of this committee are: E. B. Forbes, State College, Pa., Chairman; H. H. Mitchell, Urbana, Ill.; G. S. Fraps, College Station, Texas; F. B. Morrison, Ithaca, N. Y.; C. C. Culbertson, Ames, Iowa; E. W. Crampton, Quebec, Canada; M. Kleiber, Davis, Calif.; A. G. Hogan, Columbia, Mo.; L. A. Maynard, Ithaca, N. Y.; Max Kriss, State College, Pa.

of microorganisms inhabiting certain segments of the tract. While these losses can be measured directly only in a respiration chamber, they may be estimated for cattle indirectly from data obtainable in a simple digestion trial, and for sheep and horses when suitable prediction equations are devised. The wastage of nutritive energy in the urine completes the sum total of undigested and non-metabolizable energy, in so far as these are technically separable from the metabolizable energy. The latter unavoidably (at the present time) includes an item (the heat of fermentation) that relates to digestive wastage and that is in fact non-metabolizable.

The metabolizable energy of a feed or ration summates, with the exception just noted, the organic nutrients that actually enter into the assimilatory processes of the tissues themselves, and is expressed in terms of their common denominator, their content of stored energy, measured in heat units, that may be liberated by oxidation. The metabolizable energy is a more significant measurement of the value of a feed or ration in satisfying the energy requirements of the animal than is the sum of total digestible nutrients, because it excludes wastages of energy for which no deduction is made in the mere estimation of nutritive material of the food that does not reappear in the feces.

The nutritive significance of the content of a feed or ration in metabolizable energy is enhanced greatly by the trend of modern research in the general field of energy metabolism. In its most practical form, one of the conclusions toward which the trend of research in energy nutrition seems to be leading may be stated as follows: The utilization of the metabolizable energy of well-balanced rations approaches constancy for the same kind of animals in approximately the same condition of physiological functioning. If later research confirms the validity of this hypothesis, it would be possible to assume that the net energy of well-balanced rations is proportional to their metabolizable energy for any given combination of animal functions.

It seems to be generally recognized that available information of the digestibility of American feeds, and especially of their contents of metabolizable energy, is seriously inadequate for the purposes for which such information is applied in animal nutrition. Many of the earlier digestion trials were marred by the employment of too few experimental animals and too short experimental periods. With the progress of time, new feeds have come into use, and the processing of other feeds has changed in ways that may modify their digestibility (Kraybill, 1940), (Sievert, 1941). The result is that the data of many digestion trials are outmoded, while many new feeds and feeds processed by new methods have been inadequately studied. Improvements in the technic of digestion trials and in the accuracy of some of the pertinent chemical analytical methods have probably resulted in a situation in which

the later digestion trials should in general receive greater weight than the earlier. Consultation of one of the latest compilations of the results of digestion experiments (Morrison, 1936) reveals the fact that lespedeza hay, for example, has not been studied at all, while information on cereal hays, fresh grasses, peanut oil meal, tankage and wheat bran, with reference to ruminant digestion, is either non-existent or probably inadequate.

This subcommittee believes that one of its most important functions is to call attention again to this situation, and to solicit the cooperation of animal nutrition laboratories throughout the country in remedying it. The work to be done is not entirely a routine one, although adequate methods of conducting digestion trials are at hand and adequate apparatus has been described in the literature. There are interesting and important problems yet to be solved with reference to the factors determining and influencing the digestive processes. The most efficient use of the results of digestion experiments and the best methods of planning such experiments to yield the most significant results, await the solution of these problems. In other words, what is needed is true research, especially in the field of ruminant digestion.

The report will continue with a discussion of some of these problems and with recommendations with reference to their solution where recommendations seem to be justified. These problems are all statistical in character, since the phenomena measured exhibit at times such great variability, even among animals of the same species and under the same conditions of ration and management. While a number of animals should be used in digestion experiments, this need not discourage laboratories with limited factilities, since the experiments need not be run simultaneously. Also, developments in experimental technic indicate that the time required for digestion trials, the labor cost, and even the need for special equipment may possibly be reduced by the use of an unabsorbable reference substance, such as ferric oxide, chromic oxide, silica or lignin, either mixed with the ration or, in the latter two cases, naturally present there. By determining the ratio, in both feed and feces, of the nutrients of interest to the reference material, the corresponding digestion coefficients can be readily computed. In this type of experiment, quantitative collections of feces are not required, and the method may be applied even to animals on pasture by devising suitable methods of obtaining uncontaminated samples of feces. Reports on the accuracy of the method have been diverse, some being distinctly favorable (Bergeim, 1926) (Hamilton *et al.*, 1927–1928) (Gallup and Kuhlman, 1931) (Heller, Breedlove and Likely, 1928), while others suggest a limited applicability (Gallup and Kuhlman, 1936) or a serious sacrifice in accuracy (Knott, Murer, and Hodgson, 1936) as compared with the ordinary method of measuring digestibility.

Feeds That Should Be Studied

In studying many, if not most, of the problems to be discussed, there is a wide latitude in the choice of feeds to use. Hence, it is recommended that, in so far as possible, those feeds be selected for study that are of most economic importance in the agricultural region in which the laboratory is located, and that they be combined into rations, in so far as possible, in accordance with good feeding practice. The lack of information with reference to the digestibility of new feeds of promising importance may justify a departure from this recommendation.

Species of Animals to Use

Obviously direct information on digestibility of feeds and rations is desired for each class of livestock. It is recommended, however, that attention be concentrated upon ruminants, because of their great economic importance to agriculture, and because the losses of nutrients in digestion are relatively so large, probably a result of the nature of the rations they consume, and so variable, because of the peculiarities of ruminant digestion.

In comparing the digestive powers of different species of animals, the plane of nutrition should be given serious consideration, since digestibility will tend to vary, with some rations at least, as the daily allowance of feed is decreased or increased. The plan followed by Forbes *et al.* (1937), in their study of the relative digestive efficiencies of cattle and sheep, of comparing levels of feeding at maintenance or equal multiples of maintenance, seems a reasonable one.

It may be possible to find suitable laboratory animals that may be used as "pilot" animals in place of the larger farm animals, thus obviating any need of expensive equipment and high labor costs. Crampton, Campbell and Lange (1940) did not find that the rabbit was a successful substitute for a ruminant in digestion work, but possibly the laboratory rat can substitute for the pig in this respect. It would seem to be worth while to investigate such possibilities further.

Nutrients to Study

Digestibility studies are usually carried out with reference to all of the classes of nutrients involved in the ordinary routine chemical analysis of feeds. Serious objections to this system of analysis have been made, especially with reference to the subdivision of carbohydrates into crude fiber and nitrogen-free extract (Crampton and Maynard, 1938). The apparent digestibility of both of these subdivisions is for ruminants, and possibly for horses also, fictitiously high, since the gaseous products of carbohydrate fermentation are included with the truly digestible products. The error thus incurred may be enormous (Fries, 1922) (Krogh and Schmit-Jensen, 1920). The ap-

parent digestibility of ether extract, for rations containing large amounts of roughage, is of little significance, not only because the determination itself on this class of feeds includes a preponderant proportion of substances having no relation to fats and little nutritive value, but also because for rations low in ether extract the metabolic products of the feces may account for much the greater part of the total ether-soluble material. The apparent digestibility of mineral matter is of no certain significance, since the fecal mineral matter may include relatively large amounts of mineral substances excreted into the intestinal tract from the blood.

The main purpose of determining separate coefficients of apparent digestibility for the various classes of nutrients recognized in the usual routine chemical analysis is to secure values for the contents of digestible crude protein and of total digestible nutrients in the feeds and rations studied. However, the metabolizable energy content of a feed is a more significant value than the content of total digestible nutrients and is determinable by procedures less open to criticism.

It is recommended, therefore, that digestion studies should include, as a minimum, measurements of the apparent digestibility of nitrogen (crude protein) and of the content of metabolizable energy. If there are reasons for extending consideration to other measurements, it would seem to be more profitable to study the digestibility of nutrients, or classes of nutrients, better characterized and more accurately determinable chemically than those involved in the old routine method of analysis. Such additional measurements might well include lignin, cellulose, fatty acids, and, for non-ruminant experimental subjects, available carbohydrates determined by some such procedures as those proposed by McCance and Lawrence (1929).

It is recommended that the metabolizable energy of feeds be determined by means of a bomb calorimeter instead of by the use of factors applied to the various classes of nutrients.[2] Such determinations may be readily and accurately carried out upon feed, feces and urine by an instrument that is not prohibitively expensive. For non-ruminants, exclusive of the horse, the metabolizable energy may be considered without appreciable error to equal the difference between the gross energy (heat of combustion) of the feed and that of the urine and feces combined. For ruminants and for horses the energy content of combustible gases also must be considered. For all practical purposes, the fermentation gases consist only of methane and carbon dioxide, of which only the former is combustible. While the determination of the amount of methane produced by an animal requires the use of a respiration chamber, it may be calculated for cattle with satisfactory ac-

[2] However, Fraps (1940) claims that the heats of combustion of rations and excrement for chickens can be estimated by the use of appropriate factors applied to the chemical constituents in these materials.

curacy from the amount of total carbohydrate digested (Bratzler and Forbes, 1940), or from the amount of total dry matter consumed (Kriss, 1930). For rations consisting of roughage only, Kriss proposes the following prediction equations:

$$y = 0.0198x + 9 \quad (1)$$

and for mixed rations,

$$y = 0.0225x + 18 \quad (2)$$

in which y is the methane produced in grams daily, and x is the total dry matter consumed daily in grams. It is well worth while to secure data from which similar equations can be derived for the sheep and the horse.

The recommendation that digestion studies may be restricted to a determination of the digestibility of nitrogen (crude protein) and of the content of metabolizable energy does not imply that a complete chemical analysis of all feeds tested need not be made. On the contrary, such analyses by the best practicable chemical methods available are imperative in such experiments for the purpose of describing the feeds in the most complete manner. Variations in the chemical composition of the same grade of the same feed may be of significance in determining the results of the experiment, since there is a close correlation between the digestibility of feeds and rations and their content of certain chemical constituents.

Significance of Refused Feed in Digestion Experiments

The refusal of feed by experimental animals during a digestion trial is generally indicative merely of the fact that more feed was offered than the animal cared to consume. In such a case, especially if the refusal of feed occurs at the beginning or the end of the trial, the accuracy of the results obtained is impaired, because the feces collected may not be representative of the feed consumed. By analyzing the orts and deducting the nutrients contained in them from the nutrients offered, the possible error may not be entirely eliminated if the orts are not representative of the feed consumed.

If the refused feed is inedible and indigestible material that animals will consistently refuse, such as the coarse woody stems of soybean hay, the nutrients (and energy) contained in the orts might better be added to the nutrients (and energy) in the feces rather than be subtracted from these offered, in calculating digestion coefficients and metabolizable energy values. If they are handled in the ordinary way, the digestion coefficients and metabolizable energies obtained, when applied to the hay as offered, may considerably overestimate the digestible nutrient content and the content of metabolizable energy. This subject has been discussed in some detail by Hamilton *et al.* (1928).

Effect of the Plane of Nutrition

The effect of the plane of nutrition on the digestibility of feeds and rations and on their contents of metabolizable energy seems to be much more evident with mixed rations than with all-roughage rations and therefore may be largely a phenomenon of ruminant nutrition. Whether it occurs with horses has not been determined.

Where the effect occurs, a depression of digestibility and metabolizability of nutrients accompanies an increase in the plane of nutrition. This depression does not affect all nutrients equally; apparently the carbohydrates and ether-soluble constituents are the more constantly affected and to the greater extent. However, no general statements as to the effects of the plane of nutrition on the digestibility of feed nutrients and the metabolizability of their contained energy can hope to go unchallenged, because so little is known about them. In particular the underlying causes for these effects are quite obscure, so obscure that it seems impossible, at the present time, even to formulate a working hypothesis.

The importance of the problem is obvious and may be illustrated by the general belief that published digestion coefficients are too high for use at productive levels of feeding by at least 5 percentage units, simply because they have in general been obtained at maintenance, or only slightly supermaintenance, levels of feeding.

It is recommended that the effects of plane of nutrition on the digestibility of rations and their contents of metabolizable energy be studied thoroughly and systematically. The first and most urgently desired objective would be a mathematical description of these effects in the case of rations of practical significance in livestock feeding, whereby they may be predicted from the plane of nutrition. The ultimate objective, of course, would be a determination of the causative factors and relations involved.

Associative Effects of Feeds in Digestion

The indirect determination of the apparent digestibility of concentrate feeds that cannot be fed alone, by adding them as supplements to basal rations whose digestibility has been previously determined, has frequently yielded impossible coefficients, either negative in significance or greater than 100. Such coefficients are especially likely to be obtained for those nutrients occurring in small amounts in the supplemental feed and in relatively large amounts in the basal ration. Such effects are called "associative" effects, and have been obtained almost exclusively with ruminant animals. As revealed in non-ruminant animals by comparing directly observed coefficients for a feed when fed alone with indirectly obtained coefficients (Dietrich and Grindley, 1941) (Grindl Carmichaeley, and Newlin, 1917), the influence

of technical and biological errors must be ruled out before true associative effects can be established. This is difficult to do, because indirectly determined coefficients, being based upon "difference" calculations, are subject to all of the errors and deviations resulting from the attempt to duplicate conditions in the two digestion trials.

Associative effects of feeds are evidently the results of multiple causative factors, since the nutrients affected vary, although the carbohydrates seem to be always involved, and the magnitude of the effects varies also. They are generally quite unpredictable as they occur in the mixing of natural feeds. However, in certain simplified experiments the causes of the observed associative effects seem clearly evident. When glucose is added to a basal ration containing roughage, it has recently been shown (Mitchell, Hamilton and Haines, 1940), in confirmation of earlier experiments, that the digestibility of the crude fiber of the basal ration by cattle is markedly depressed. Similar effects of starch and molasses, other forms of carbohydrate readily available to paunch microorganisms, have been reported in ruminant feeding, although some exceptions could be cited. The reason for this depressing effect seems obvious: The microorganisms attack the more readily available carbohydrates and leave the fibrous constituents of the basal diet, containing celluloses and hemicelluloses, unfermented or less extensively fermented than if the soluble sugars and starches were not present.

Again, when a nitrogenous compound like urea is added to a basal low-nitrogen roughage-containing diet, the digestibility of cellulose has been shown to be greatly improved (Harris and Mitchell, 1941). In the absence of nitrogenous compounds from the ration or in the presence of only small amounts, the growth of the normal flora and fauna in the paunch must be greatly inhibited as this unphysiological regime is continued, ultimately leading to serious digestive disorders (Miller, 1937). The higher carbohydrates constituting the cell walls of the plant material are not ruptured and dissolved and their contained nutrients may not be rendered accessible to the animal enzymes of the abomasum and the intestinal tract. The situation is remedied when nitrogenous food is again provided for the growth of the paunch microorganisms.

The depressing effect of pure carbohydrates on the apparent digestibility of protein may be a true associative effect to the extent that it is an indirect result of the effect on the fibrous constituents of plant food, rendering the contained protein less accessible to the animal proteases. However, most of this so-called depression in the digestibility of protein brought about by carbohydrate food is purely fictitious, being due merely, to an increased excretion of metabolic nitrogen in the feces (Mitchell, 1926). An excellent discussion of these and other associative effects of feeds in digestion has been given by Armsby (1917).

Considering the fact that the pronounced associative effects of feeds in digestion have been noted only with ruminants and the fact that innumerable possibilities suggest themselves as to how feeds may modify the growth and activities of the paunch microorganisms, and indirectly the digestibility of the nutrients on which they grow (and other nutrients also), it is an attractive theory that such associative effects are all traceable to events occurring within the ruminant paunch. The digestive enzymes are remarkably efficient hydrolytic catalysts and generally the reactions that they promote will proceed to completion in the course of the passage of food through the true stomach and the intestines. If, however, insoluble barriers are interposed between the enzymes and their substrates, the digestion of the latter will be incomplete. These barriers would consist of fibrous plant constituents, containing celluloses, hemicelluloses and lignin, the removal of which would depend largely on the extent to which they are fermented by the microorganisms of the paunch and other segments of the alimentary tract where stagnation of food occurs. The removal of these barriers is most effectively accomplished in the polygastric animal, and in such animals the factors determining the extent of removal will operate within the paunch, and would be concerned with the food supply of the microorganisms inhabiting it and with the conditions of temperature, moisture content, and chemical reaction of the surrounding medium. The effects of any supplementary feed upon any of the conditions determining microorganismal growth in the paunch would contribute to its associative effects in digestion, if not account for them entirely.

The importance of this problem to the practical use of the results of digestion and metabolism experiments is obvious, and it is recommended that it be given intensive study with the objective of ultimately being able to predict when associative effects will occur and their extent. The hypothesis above discussed would seem to be a reasonable one upon which future research may be based.

Relation Between the Chemical Composition of a Feed and Its Digestibility

Digestion coefficients are almost universally considered to be characteristic of the feeds for which they have been obtained, subject of course to variation due to plane of nutrition and associative effects with other feeds. This may not be a reasonable attitude to take, because obviously a change in the composition of a feed, especially with reference to its content of those components removable only by fermentation, may cause a change in its digestibility. Thus, the digestion coefficients obtained upon one sample of a feed cannot without error be applied to other samples of different chemical composition.

Jarl (1938) made a statistical study of 147 published determinations of the digestibility of Scandinavian hays, and was able to demonstrate a high negative correlation between digestibility and fiber content. He computed the regression of digestibility on fiber content for each nutrient separately. On the basis of these important findings, he proposed the use of the digestibility based on the fiber content in estimating the feeding value of hay by the Kellner method, instead of using average fixed coefficients of digestibility.

Axelsson (1938) demonstrated a close negative correlation between the crude fiber content of the dry matter of feeds and the digestibility of the organic substance by cattle. The regression proved to be rectilinear and was well represented by the following equation:

$$y = 90.1 - 0.879x \qquad (3)$$

in which y is the digestibility of the organic substance of the feed or ration and x is the crude fiber content on the dry basis. Axelsson (1940) discusses this important relationship further.

These reports of Jarl and Axelsson seem sufficiently important to the objectives of this report to warrant a study along similar lines of the digestibility of American feeds. Accordingly, the data on the digestibility of feeds by rabbits, recently reported by Voris *et al.* (1940), were consulted. The digestibility of the organic matter proved to be highly correlated with the crude fiber content on the dry basis of the 45 feeds and rations tested, r being equal to -0.899. On plotting the data on coordinate paper the regression of digestibility (y) on crude fiber content (x) was clearly rectilinear and was well described by the following equation:

$$y = 96.48 - 1.551x. \qquad (4)$$

A comparison of equations (3) and (4) indicates that crude fiber depresses the digestibility of feed in the rabbit to a considerably greater extent than in cattle. In the latter case, an increase in the fiber content of a feed on the dry matter basis of 1 per cent, depresses the percentage digestibility of the organic matter by 0.879 unit, while with the rabbit, the depression is almost twice as great, i.e., 1.551 units. This is merely another way of saying that rabbits digest crude fiber considerably less efficiently than cattle. The fact that for fiberless feeds and rations, the equations predict that the organic matter would be digested to the extent of 96.48 per cent by rabbits and only 90.1 per cent by cattle can be interpreted as an indication that paunch organisms, to the extent that they have to subsist upon a fiberless pabulum, will in some way impair the apparent digestibility of the nutrients contained therein, possibly by contributing to that pabulum relatively indigestible constituents in their own cells.

A similar study on digestion in poultry, using the data of 21 experiments reported by Kaupp and Ivey (1923) and 3 experiments reported by Lenkeit and Becker (1938), again revealed a high correlation between the crude fiber content of the dry feed and the digestibility of organic substance ($r = 0.746$), a rectilinear regression, and the following regression equation:

$$y = 86.06 - 1.955x \tag{5}$$

x and y having the same significance as in equations (3) and (4). Comparing equation (5) with (4), it appears that crude fiber is digested to a less extent by poultry than by rabbits, since an increase of 1 per cent in the fiber content of the dry matter consumed is associated with a decrease in the percentage digestibility of the organic matter of 1.95 units, instead of 1.55 units.

It is an attractive theory that differences in digestive efficiency among different species of animals relate almost entirely to the efficiency with which animals break down the fibrous, woody constituents of the feed by means of the symbiotic relationship existing with the microorganisms inhabiting the digestive tract. If this is true, then equations of the type represented by those just discussed may describe in a useful and satisfactory manner the differences in digestive efficiency existing among species of animals and permit the prediction of the digestibility of feeds for which direct experimental data are not available.

It is recommended that this line of attack be more widely applied to the results of American digestion trials. Studies of the relationship of the crude fiber content of feeds to the digestibility of individual nutrients would be well worth while. Possibily also some determinant of digestibility possessing more significance than crude fiber or susceptible to more accurate chemical analysis may be found. As an indication of what might be done along these lines, the results of 60 metabolism experiments on chickens reported by Fraps *et al.* (1940) have been studied with reference to the relationship between the crude fiber content of the rations fed, computed on the dry basis, and the percentage metabolizability of the gross energy. The correlation was negative and high, r being -0.911. The regression of the latter factor on the former is rectilinear and the regression equation is

$$y = 86.90 - 3.624x \tag{6}$$

in which y is the percentage of the gross energy of the feed that is metabolizable and x is the crude fiber content on the dry basis. The equation indicates that the gross energy of fiberless rations is on an average 86.90 per cent metabolizable, and that for each increment of 1 per cent in the crude fiber content of the dry feed there is a depression in the percentage metabolizability of the gross energy of 3.62 units.

A relationship between the apparent digestibility of protein and the protein content of the feed was pointed out by Stohmann (1869). He devised a prediction equation which, in its simplest form, may be written

$$P' = \frac{P}{1 + \frac{S}{9P}}$$

in which P is the amount of protein consumed, P' is the amount of protein digested, and S is the sum of the nitrogen-free organic constituents consumed. According to this equation, as the proportion of protein consumed is increased from 5 per cent to 50 per cent, the apparent digestibility of protein increases from 32.1 to 90.0 per cent.

Mitchell (1926) has discussed this relationship and has shown that it results from the factors governing the excretion of metabolic nitrogen in the feces. Apparently the main factor in determining the amount of metabolic nitrogen excreted in the feces, for intakes of food equivalent to or greater than a maintenance ration, is the amount of dry matter consumed, provided that the fiber content of the dry matter does not vary greatly. This being true, the proportion of feed nitrogen to metabolic fecal nitrogen will increase as the proportion of feed nitrogen to dry matter in the feed increases. This fact will cause an increase in the apparent digestibility of protein (nitrogen) as the protein content of the dry matter increases. Mitchell (1926, tables 7 and 8) has shown that there is a striking positive correlation between the reported coefficients of apparent digestibility of protein of feeds and their contents of crude protein on the dry matter basis, and that this correlation does not seem to be primarily dependent on the fact that low-protein feeds are quite often low in crude fiber also. The calculations there exhibited reveal the fact that even in ruminants the metabolic products in the feces are so considerable as to be the main factor in determining the apparent digestibility of protein. In agreement with this finding, and in confirmation of it, it is further shown that some prominent instances of the so-called depression of the digestibility of protein by carbohydrate can be entirely explained on the basis of the extra metabolic fecal nitrogen thus produced. In ruminants, and probably in other animals also, the fecal nitrogen resulting from digestion is generally determined largely by the dry matter consumed, and not by the actual digestibility of the feed protein.

A statistical study based upon the digestibility data summarized by Morrison (1936) reveals that the relationship between the protein content of a feed on the dry basis and the apparent digestibility of its protein is not a rectilinear one, but a hyperbolic one. For roughages, for which the digestion coefficients were presumably directly determined, the relationship, for

feeds containing 5 per cent or more of protein on the dry basis, is represented by the following equation:

$$D = 42.64\ (P-5)^{.215} \qquad (8)$$

in which D is the apparent digestibility of the protein and P is the protein content on the dry basis. The agreement between the observed digestibilities and the predictions by the above equation is illustrated by the following tabulation:

Protein content of feeds on dry basis		Number of feeds in class	Apparent digestibility of crude protein	
Range	Assumed average		Observed average	Predicted
6– 8	7	36	50.9	49.5
8–10	9	32	54.9	57.4
10–12	11	13	61.4	62.6
12–14	13	20	67.9	66.6
14–16	15	22	72.0	69 8
16–18	17	18	71.8	72.7
18–20	19	13	74.8	75.1
20–22	21	5	77.8	77.3
22–24	23	4	79.3	79 3

It may be stated that equation 8 gives consistently higher predictions of protein digestibility than does equation 7, although the two may not be so directly comparable due to the different bases on which the predictions are made.

In view of the overwhelming evidence of a close correlation between (1) the content of feeds in fibrous constituents and the apparent digestibility of the organic matter, as well as the metabolizability of the gross energy, and (2) the crude protein content and the apparent digestibility of the crude protein, it is recommended that the digestion coefficients of feeds and rations and the contents of metabolizable energy be considered, not as constant characteristics of those feeds and rations, but as measurements indissolubly connected with the particular samples with which they were obtained. They must be corrected for differences in chemical composition if applied to other samples of the same feeds and rations, as Axelsson (1940) has illustrated.

It is believed that the relationship between the chemical composition of feeds and their digestibility and contents of metabolizable energy is a fertile field of study. The possibility that the digestibility of all feeds and the metabolizability of their gross energy may ultimately be satisfactorily predictable from generalized equations of the types illustrated is also recog-

nized. In other words, the digestibility of one feed may differ from that of another, not because of any physical or chemical properties peculiar to each, but because of different contents of well-recognized constituents, particularly as some of these constituents (the indigestible polysaccharides) diminish the opportunity for the digestive enzymes to come in contact with their respective substrates. The apparent digestibility of protein (nitrogen) depends, for all feeds and in a similar manner, on the ratio existing between protein and non-protein constituents. The inconsiderable effects of other factors is illustrated by the fact that the method of preparation of feed (soaking, drying, ensiling, chopping or grinding) is generally without appreciable effect on digestibility in the case of animals retaining their full digestive powers.

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NEWS AND NOTES

Members are reminded that the thirty-fifth annual meetings of the American Society of Animal Production will be held in Chicago on November 27–29, 1942. Titles and abstracts of papers to be presented at these meetings *must* be in the hands of the Secretary, Dr. A. D. Weber, Kansas State College, Manhattan, Kansas, by September 15. Titles received after this date cannot be included in the program and abstracts cannot be published. The abstracts should be typed in the form used for abstracts in the February 1942 issue of the JOURNAL and must not exceed 250 words (usually one page or less, double spaced). *Please check items 5 and 6 of "Information for Contributors."* Chairmen of the sectional meetings are:

Animal breeding	A. B. Chapman, Wisconsin
Animal industries	F. M. Simpson, Swift and Co. (Chicago)
Artificial insemination	C. E. Terrill, U.S.D.A. (Dubois, Idaho)
Beef cattle	J. H. Knox, New Mexico
Dairy cattle	W. E. Krauss, Ohio
Extension	W. W. Derrick, Nebraska
Horses and mules	L. H. Blakeslee, Michigan
Meats	L. E. Kunkle, Ohio
Nutrition	C. L. Shrewsbury, Indiana
Pasture	W. P. Garrigus, Kentucky
Sheep and wool	R. F. Cox, Kansas
Swine	B. W. Fairbanks, Illinois
Teaching	F. L. Bentley, Pennsylvania

Contributors are asked to read carefully the "Information for Contributors" on the back cover. Item 3 should be checked carefully before a manuscript is typed in final form. Please note that lines must be numbered consecutively, beginning with "1," on each page of text.

The Southern Section of the American Society of Animal Production met in February in connection with the meetings of the Southern Agricultural Workers. The officers elected for the current year were J. C. Grimes of Alabama Polytechnic Institute, Chairman, and E. G. Godbey of Clemson Agricultural College, Secretary.

The Western Section of the American Society of Animal Production will meet at the University of Wyoming at Laramie on June 16 and 17, 1942. The theme of the meetings will be "The Production of Food and Fiber for War." Officers of the Western Section are R. T. Clark of Montana, President, J. H. Knox of New Mexico, Vice-President, and F. S. Hultz of Wyoming, Secretary.

The North Atlantic Section of the American Society of Animal Production will hold its annual summer meeting at the U. S. Morgan Horse Farm, Middlebury, Vermont, on June 5 and 6, 1942. The various state institutions will report on their recent work, and experimental work under way at the U. S. Morgan Horse Farm will be inspected. Myron Fuerst of Pine Plains, N.Y. is president of the North Atlantic Section, and V. A. Rice of Amherst, Massachusetts is Secretary.

The first issue of Section F of Biological Abstracts appeared in January 1942. This new venture is designed to meet the needs of men engaged in the animal industries. Section F will contain all of the abstracts published in the parent edition of Biological Abstracts that have to do with the breeding, nutrition, metabolism, husbandry, reproductive and other physiology, anthropod pests of livestock, poultry, and semidomesticated animals and birds, including pet stock. The literature which is of interest to animal husbandmen is much more widely scattered throughout the biological publications than is generally assumed. For example, the 514 abstracts included in the January issue of Section F are drawn from a total of 135 journals. Biological Abstracts now abstracts the literature from a total of 1575 periodicals, so subscribers

to the new section will be assured a wide scope of information. Section F will consist of 10 issues per year and subscribers will receive the index to the complete edition of Biological Abstracts. The subscription rate is $5.00 (foreign $5.50). Inquiries should be sent to Biological Abstracts, University of Pennsylvania, Philadelphia.

Raymond S. Glasscock, formerly of the staff at North Carolina State College, has recently been appointed associate animal husbandman in charge of Animal Husbandry Investigations, University of Florida, and began his duties there February 1.

George M. Sidwell, a graduate of Utah State Agricultural College, and L. Otis Emik, a graduate of Stanford University and formerly graduate assistant in genetics at the University of California at Davis, are now employed as junior animal husbandmen, assisting with work in genetics and physiology, at the Western Sheep Breeding Laboratory and U. S. Sheep Experiment Station, Dubois, Idaho.

Henry R. Keller, a graduate of the University of Arizona and formerly with the Tovrea Packing Co., Phoenix, Arizona, is now employed as a junior animal husbandman, assisting with wool investigations, at the Western Sheep Breeding Laboratory and U. S. Sheep Experiment Station, Dubois, Idaho.

Alma C. Esplin and Harry H. Smith have been transferred to full time Extension work at the Utah State Agricultural College. Previously these men have held joint positions with the Extension Service and with the School of Agriculture, or with the Agricultural Experiment Station.

Geo. F. Ellis, extension animal husbandman, New Mexico College of Agriculture, has resigned to become assistant manager of the Diamond A Cattle Co., with headquarters at Roswell, N. Mex. He is succeeded by Stephen L. Brock, a graduate of the Texas Technological Institute, who has been a county agricultural agent in New Mexico.

Glynden T. Easley, research assistant in animal husbandry at the University of Missouri, sailed for Peru on March 2. He will spend six months in Peru, working on an artificial insemination project.

Hugh C. McPhee, Chief of the Animal Husbandry Division, U. S. Bureau of Animal Industry, is on temporary detail as assistant to E. C. Auchter, who is Agricultural Research Administrator in the U.S.D.A.

Harry L. Garrigus, emeritus professor of animal husbandry, University of Connecticut, was extended a testimonial banquet, including the presentation of his portrait to his alma mater, by a host of friends, colleagues, and leaders of New England agriculture on February 1, 1942, at Storrs.

H. W. Marston of the Office of Experiment Stations, U.S.D.A., is on temporary detail as assistant to E. C. Auchter, Agricultural Research Administrator in the U.S.D.A.

E. W. Sheets is now secretary of the U. S. Livestock Association with headquarters in Washington, D. C.

R. M. Denhardt of the staff of the Texas A. & M. College, and secretary of the American Quarter Horse Association, has accepted a special assignment from the U. S. Department of Agriculture in cooperation with the Department of State to make a survey of livestock conditions in Brazil. His headquarters in that country will be Porto Alegre, Rio Grande de Sul.

Fred H. Leinbach, head of the animal husbandry department, University of Maryland, was elected president of the National Block and Bridle Club at the annual meeting held in connection with the 1941 International Live Stock Exposition.

James A. Whatley, Jr., assistant professor of animal husbandry, Oklahoma A. & M.

College, has been called to active duty as a reserve officer in the U. S. Army and has been succeeded by Chas. S. Hobbs.

Grady Sellards, extension swine specialist, University of Kentucky, has been called to active duty as a captain in the Army Remount Service and is now on duty at the Quartermaster Depot, Front Royal, Va.

A. O. Rhoad, superintendent of the U.S.D.A. Iberia Livestock Experiment Farm, Jeanerett, La., returned in early March from a flying trip to Brazil where he acted as judge in a major cattle show.

A. O. Shaw, associate professor of dairy husbandry, Kansas State College, has been made head of the Animal Husbandry Department, North Carolina State College of Agriculture.

H. O. Hetzer, associate animal husbandman in the U. S. Bureau of Animal Industry, is now on military leave and is in the U. S. Navy doing special statistical work in Washington, D. C.